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Effect of dietary glycolytic inhibitors on pork quality

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Effect of dietary glycolytic inhibitors on pork quality

by

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ABSTRACT

Four studies were performed to determine the effectiveness of short term feeding of several dietary glycolytic inhibitors on the quality of pork. In this series of studies oxalate, quercetin, vitamin C, and zinc, naturally occurring compounds, were used shortly prior to slaughter to reduce glycolytic rate and improve subsequent pork quality. In the first experiment the oxalate was provided to 110 kg pigs in the form of sodium oxalate at 0, 7.0, and 9.7 g/ pig (110kg BW). The ingestion of sodium oxalate decreased the rate of muscle pH decline and lowered the pork water loss (WL). In an additional experiment with sodium oxalate, 30 kg pigs were used as a model for market pigs. The sodium oxalate (0 and 2.6 g/ pig) again decreased the rate of muscle pH decline postmortem and lowered pork WL.

In a second experiment the effect of dietary quercetin, an inhibitor of lactate dehydrogenase, on pork quality was determined in 110 kg pigs. Quercetin was provided at 0, 1.07, and 4.68 mg/ pig (110 kg BW). The ingestion of quercetin decreased the rate of muscle pH decline and improved the subsequent pork WL. The redness of the pork was lower with ingestion of dietary quercetin.

The last two experiments both involved using the 30 kg pig as a model for the market pig. In the third experiment high doses of vitamin C, a metabolic precursor of oxalate, was fed at 0, 290, and 704 mg/ pig. The ingestion of vitamin C decreased the rate of postmortem muscle pH decline and lowered subsequent pork WL. The ingestion of vitamin C also improved the red color score of pork products and reduced the paleness of the pork products.

In the forth experiment, high levels of dietary Zn were provided shortly prior to slaughter. Dietary Zn was fed at 0, 4.9, 7.8, and 11.8 g/ pig. The ingestion of Zn did not

alter the rate of postmortem muscle pH decline and did not improve pork WL. The ingestion of dietary Zn did tend to improve the redness color score of pork.

CHAPTER 1. GENERAL INTRODUCTION

Meat quality has become a concern in the pork industry. The qualities of a pork product important to consumers are color, purge, and texture of the meat (NPPC, 1994). These characteristics influence whether consumers will buy a pork product when viewed in a meat case. Meat quality is important because consumer demands for a product must be addressed for pork to maintain and expand its market share.

These quality traits also influence the frequency of pork purchases (NPPC, 1994). Water retention affects the juiciness and tenderness sensations of the cooked product. The perceived texture of the product can also influence the desirability of the product. These qualities are also influencing the consumer preference for future meat selections. Thus, these traits impact the value of the product in several ways. The water and protein losses associated with purge lower the amount of saleable product because meat is sold by weight. These same losses impact the ability of the retailer to sell the meat products to the consumer as well because of appearance.

The inhibition of glycolysis shows potential for improving the pH decline in postmortem muscle and improving the subsequent meat quality (Klont et al., 1994). The application of a glycolytic inhibitor prior to slaughter in a non-intrusive manner would be preferable to injection or other intrusive application. The risk of contamination from oral application would be lower than other methods of application.

Dissertation organization

The dissertation is divided into a literature review, four papers, and a general conclusion. The papers were prepared in a style appropriate for submission to the *Journal of Animal Science*.

Literature Cited

Klont, R.E., E. Lambooy, and J.G. van Logtetijn. 1994. Effect of dantrolene treatment on muscle metabolism of anesthetized pigs of different halothane genotypes. J. Anim. Sci. 72:2008-2016.

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CHAPTER 2. REVIEW OF LITERATURE

Consumer Appeal

Meat quality has become a concern in the pork industry. The qualities of a pork product important to consumers are color, purge, and texture of the meat (NPPC, 1994). These characteristics influence whether consumers will buy a pork product when viewed in a meat case. Meat quality is important because consumer demands for a product must be addressed for pork to maintain and expand its market share.

These quality traits also influence the frequency of pork purchases (NPPC, 1994). Water retention affects the juiciness and tenderness sensations of the cooked product. Pork products with higher purge will have lower water retention (Hedrick et al., 1994). The perceived texture of the product can also influence the desirability of the product. These qualities are also influencing the consumer preference for future meat selections. Thus, these traits impact the value of the product in several ways. The water and protein losses associated with purge reduce the amount of saleable product because meat is sold by weight. These same losses impact the ability of the retailer to sell the meat products to the consumer as well because of appearance.

Appearance

Color

The color of pork can range from a dark red to a pale gray color (Offer and Knight, 1988). The color preferred by most consumers is a pink color (NPPC, 1994). The amount and form of myoglobin present which determines the redness of pork (Lawrie, 1985). Pork

with more myoglobin has more red color, whereas meat with less myoglobin is paler in color (Offer and Knight, 1988).

Two additional factors that impact color are amount of drip loss and retention of sarcoplasmic protein of which myoglobin is prominent (Offer and Knight, 1988). The exudate that is collected from pork is generally red in color because of the loss of myoglobin from the sarcoplasm. The red fluid is not blood as is a common perception. Hence, factors that reduce the formation of drip loss also result in greater retention of myoglobin. The denaturation of these proteins will also influence whether they are retained. Denatured myoglobin does not maintain its ability to influence color due to its resultant loss in functionality (Hedrick et al., 1994).

Water loss

Water loss is a term for the fluid that accumulates in a container when meat is on display (NPPC, 1994). This accumulation of fluid detracts from the visual impression of pork (Offer and Knight, 1988). The visual impact of purge on the consumer is related to the volume and color of the fluid expelled. The consumer prefers pork that has lower versus higher amount of fluid accumulation. A cut of meat sitting in a pool of red fluid is generally not acceptable to consumers (NPPC, 1994).

The volume of fluid expelled from meat is related to the amount of water expelled from the intracellular space of the muscle as it is converted to meat in the post-mortem period (Offer and Knight, 1988). This expulsion of fluid is influenced by several factors that will be discussed in greater detail later: such as myosin denaturation, time of rigor mortis development, and proximity of the muscle to the isoelectric point of its proteins (Offer, 1991;

Hedrick et al., 1994; Xiong and Blanchard, 1993). The expulsion of fluid can be a problem even in meat that maintains a normal color: a condition known as red, soft, and exudative (Warner et al., 1997). These researchers report differences in the amount and types of proteins denatured between red, soft, and exudative (RSE), red, firm, and normal (RFN), and pale, soft, and exudative (PSE) classes of pork.

The color of the fluid expelled from the meat is related to the amount of sarcoplasmic protein denatured as previously discussed. More myoglobin in the fluid results in the accumulation being redder and less attractive to the consumer of the pork product (Offer and Knight, 1988; NPPC, 1994). The stability of the proteins responsible for the red color in meat may be more stable than those associated with the exudative condition as is demonstrated by the existence of PSE, RSE, and RFN classes of pork. These observations will be more fully described later in a discussion of protein denaturation.

Texture

Texture, as it relates to the visual appearance of pork, impacts the acceptability because consumers do not prefer meat with a filmy sheen (NPPC, 1994). This sheen is closely related to the amount of exudate formed. These differences are demonstrated by the condition of RSE versus RFN meat. The RSE meat which has sheen is likely to lose 50% more water than does RFN meat that has a normal appearance (Warner et al., 1997).

Eating Characteristics

Juiciness

Juiciness and tenderness of pork have impacts on the repeated purchases of pork. These characteristics can be related to critical events as the muscle becomes meat or to the

preparation of the product (Hedrick et al., 1994; NPPC, 1994). Education on safe and proper preparation of pork should help address problems related to improperly prepared pork (NPPC, 1994). The problems associated with the conversion of muscle to meat could also be improved.

Juiciness is the perception of moisture present when meat is masticated. This is important because the palatability of meat is influenced by this characteristic (Hedrick et al., 1994). The juiciness may also impact the perception of tenderness of a meat product. The two main influences on juiciness are fat and water in the meat (Hedrick et al., 1994). Because of the advantages of growing leaner animals (improved growth rate and improved efficiency), it is unlikely the amount of fat in pork will increase. The lower fat content of pork results in water playing an increased role in the sensation of juiciness in pork. Meat with lower drip loss is juicier when cooked (Lesiak et al., 1996).

Tenderness

Tenderness is not generally as much of a problem for pork quality as the color, water holding, and texture aspects of pork (NPPC, 1994). This is most likely because of the relatively young age at which pigs are slaughtered. This does not allow for the extensive development of connective tissue in the muscle (Fang et al., 1999). The collagen in young animals does not contain as many cross links as in older animals (Fang et al., 1999; Offer and Knight, 1988).

Another important consideration in the tenderization of meat is enzymatic break down of structural proteins of the muscle cells (Koohmaraie, 1994). Several enzyme systems have been investigated for their possible roles in tenderness, these include the calpains and

cathepsins (Koohmaraie, 1994). Enzymes have environmental optimal conditions under which they are most active (Murray et al., 1996). The enzymes, their optimal environments, and their relationship to quality will be discussed later.

Conversion of Muscle to Meat

Biochemical Changes

When the animal is exsanguinated and ceases to respire, the metabolic process is shifted from an aerobic to anaerobic type of system. This shift occurs because the blood flow is interrupted, and the lungs are no longer functioning to transfer carbon dioxide and oxygen. As oxygen is rapidly depleted and carbon dioxide accumulates, oxidative phosphorylation, the method of ATP generation by mitochondria, is not able to proceed (Murray et al., 1996). The electron transport chain cannot accept electrons because oxygen, the terminal electron receptor, is not present, causing NADH to accumulate. The accumulation of NADH causes the citric acid cycle (TCA) to stop functioning rapidly after the oxygen supply is depleted.

The glycolytic pathway continues to function, to supply ATP to cells, and to allow structural and some functional characteristics of tissues and organs to be maintained for a period of time after exsanguination (Hedrick et al., 1994). Glycolysis in muscle continues until glucose, stored as glycogen, is depleted (Murray et al., 1996). Because the TCA cycle is no longer functioning to utilize the pyruvate produced, the pyruvate is converted to lactic acid to provide NAD^+ for continued glycolysis. As lactic acid accumulates post-mortem, the pH in the muscle declines (Hedrick et al., 1994). The decline in muscle pH is a key occurrence in the conversion of muscle into meat.

The glycolytic pathway functions in the absence of oxygen in much the same way it fuels movement in muscles that are overexerted. The glycolytic pathway continues to provide chemical energy (ATP) to the muscle even though oxygen is not available. Pyruvate cannot accumulate because of the need for NAD^+ for the conversion of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate (Murray et al., 1996). The NAD^+ needed for the reaction is formed during anaerobic glycolysis via the conversion of pyruvate to lactate. When the animal is alive, the control enzymes for glycolysis are hexokinse, phosphofructokinase, and possibly pyruvate kinase (Murray et al., 1996).

Hexokinase is controlled by the amount of substrate available (Murray et al., 1996). There is glucose available because of the breakdown of glycogen in the post-mortem animal. Phosphofructokinase is controlled through a series of intermediate reactions by the amount of ATP compared with the amount of ADP (Murray et al., 1996). The amount of ATP compared with amount of ADP will be low in the post-mortem animal because of the inability of the TCA to regenerate ATP; therefore, this enzyme system also will be up-regulated. Pyruvate kinase is thought to be controlled in a manner related to phosphofructokinase and thus will also be up-regulated.

Microenvironment

Upon the death of the pig, blood flow is lost. The interorgan flow of metabolites no longer occurs. Any metabolite produced postmortem will remain relatively close to the site of production. The body ceases to function in a coordinated manner. Each cell or tissue acts independently and without communication between other cells or tissues.

The cells of a living organism are in a condition known as homeostasis, a condition that is not at equilibrium and is thus termed disequilibrium (Ganong, 1995). Upon the death of the animal and the conversion to a microenvironment, the cells begin change toward equilibrium (Hedrick, et al., 1994). The mechanisms needed to maintain homeostasis, i.e. ATP regeneration, ion pumping, and compartmentalization of ions either are no longer functioning or are functioning at sub-optimal levels (Ganong, 1995).

As previously discussed, ATP regeneration is impaired as a result of loss of TCA function. ATP is required for ion pumping in the cells (Murray et al., 1996). In living animals, a large portion of the maintenance requirement is for ion pumping (Baldwin et al., 1980). The Na^+/K^+ ATPase is one such pump in which transfer of sodium out of the cell and potassium into the cell is coupled with the hydrolysis of ATP (Murray et al., 1996). When the animal dies and ATP regeneration is compromised, the ions begin to move toward equilibrium by simple diffusion.

Physical Changes

Sarcomeric Shrinkage

The sarcomere, the smallest contractile unit of muscle, begins at one Z-line and extends to the next Z-line (Hedrick et al., 1994). The sarcomere consists primarily of actin thin filaments and myosin thick filaments (Ganong, 1995). The protein titin is along the thick filament whereas nebulin runs along the thin filament (Taylor et al., 1995).

Proteins have charged portions in them because of the charges associated with the individual amino acids that form them (Murray et al., 1996). These like charges result in repulsion between the myosin thick filament and actin thin filament. For every protein, there

is a pH at which the net charge of the protein will be zero (isoelectric point), and for meat this pH ranges from 5.0 to 5.5 (Murray et al., 1996; Offer and Knight, 1988). When repulsion among structural proteins is reduced, the sarcomere will shrink.

In postmortem animals, conditions exist that promote protein denaturation (Lawrie, 1985). One of the proteins that can be denatured is myosin (Offer and Knight, 1988). Myosin heads shrink from 19 to 17 nm when they are denatured (Offer, 1991). This shrinkage reduces the distance between the thick and thin filaments when myosin is attached to actin. The amount of myosin denatured influences the amount of shrinkage because the more shortened myosin heads results in pulling of the myosin heads closer (Offer, 1991). The proteins, titin and nebulin, are secondary fibers running next to the thick and thin filaments, respectively. These proteins are implicated in the tenderization process and will be discussed later. The titin and nebulin also have been implicated as secondary or template structure for the sarcomere (Huff-Lonergan et al., 1996).

Rigor Mortis

In the process of muscle contraction, ATP is required to allow for the release of the myosin head from the actin thin filament (Ganong, 1995). Rigor mortis occurs if the amount of ATP present is no longer sufficient to allow for myosin release from actin (Murray et al., 1996).

When ATP is depleted, thin and thick filaments are bound together and the amount of overlap between them can impact the space available to hold water (Offer and Knight, 1988). Muscle of the animal goes through shrinkage during rigor mortis because of the myosin and actin being "locked" together (Olsson et al., 1994).

Results of Biochemical and Physical Changes

pH decline

During anaerobic glycolysis, lactate accumulates in the muscle tissue, resulting in a pH decline (Lawrie, 1985; Offer and Knight, 1988; Hedrick et al., 1994). Quality characteristics are correlated to the decline in pH (Ahn et al., 1992; Offer, 1991; Warner et al., 1997; Warriss, 1982). Biochemical changes that occur as a result of pH decline impact meat quality by altering the charge on proteins, the rate of enzymatic activity, and the rate of protein denaturization.

Isoelectric Point

Every amino acid has a pH at which it will not have a charge (Murray et al., 1996). When these amino acids are combined by peptide bonds to form a protein the N- and C-terminal contain charges as well as amino acids with carboxyl and amino side chains. The isoelectric point of meat proteins, as previously mentioned, is between pH 5.0 to 5.5 (Offer and Knight, 1988). The isoelectric point is the pH at which the net charge of that protein is zero. Every protein in the meat has an isoelectric point; therefore, this range represents a composite of the various isoelectric points for the proteins (Murray et al., 1996).

Charges on proteins in muscle create repulsion between proteins that comprise the structure of muscle (Lawrie, 1985). Repulsion results in spaces between the proteins and water fills these spaces (Offer and Knight, 1988). When these charges are lost, the proteins no longer repel each other. The lack of repulsion results in shrinkage of the sarcomere because the actin and myosin can be in closer contact. Shrinkage of the sarcomere results in displacement of water from its intracellular position, and the water is then more readily lost

(Hedrick et al., 1994). Water loss is higher in meat that attains a pH nearer to its isoelectric point (Kuo and Ockerman, 1984). As pork approaches a pH of 5.5, the water holding capacity is reduced (Monin and Sellier, 1985).

A condition in pigs, common in the Hampshire breed, in which a higher than normal amount of glycogen is stored in muscles, resulting in a low ultimate pH (24 h) is caused by the Napole gene (Monin and Sellier, 1985). The amount of drip loss, water loss from hot to chilled carcass weight, in the carcasses of animals with a high amount of glycogen is increased 14% (Miller et al., 1998).

Enzymatic Activity

Another consequence of pH decline is alteration of enzymatic activity. Activity for a given enzyme is highest under certain physical conditions (pH and temperature optimums) (Murray et al., 1996). Enzymatic activity is most closely associated with the tenderness of meat products because enzymes are required to break down certain proteins in the muscle (Koohmaraie, 1994).

One enzyme system that has been investigated for possible effects on tenderness is the cathepsin system. Cathepsins are lysosomal enzymes that have to be released from the lysosome to have an effect on meat tenderness (Koohmaraie, 1994). If the lysosome is disrupted and cathepsins are released, the result would be cellular damage and sarcoplasmic leakage into the surrounding spaces (Ganong, 1995). This effect would have a negative impact on water holding capabilities because disruption of muscle cell integrity can lead to increased water loss (Offer and Knight, 1988).

The calpain enzyme system is currently thought to be responsible for much of the tenderization that happens post-mortem because of proteolytic processes. Calpains specifically degrade the Z-disk (Vigoreaux, 1994). The specific proteins attacked by the calpains are titin, nebulin, filamin, desmin, and troponin-T, which are proteins associated with the Z-disk (Huff-Lonergan et al., 1996). Huff-Lonergan et al. (1996) found that, in meat with higher tenderness scores, μ-calpain had degraded these proteins to a greater extent in tender meat than in tough meat.

Because it breaks the muscle fiber at the sarcomere, the proteolytic activity of the calpain system indicates that it is the most important enzyme system in meat tenderness (Koohmaraie, 1994). The pH optimum for calpain is 6.8. Therefore, the longer the post-mortem muscle was near the pH optimum, the tenderness of the resultant meat should be higher because the enzyme is more active for a longer time.

Protein Denaturization

Proteins have primary, secondary, tertiary, and quarternary structure (Murray et al., 1996). The protein must be in its native form to maintain its functional capabilities (Ganong, 1995). Under certain pH conditions, the proteins in meat can be denatured and changed from their native form, meaning that one or more of the higher order structures have been destroyed (Hedrick et al., 1994; Murray et al., 1996).

As the pH declines below 6.1, the proteins in meat are progressively more likely to be denatured (Lawrie, 1985; Warriss and Brown, 1987). The equation $k = k_0 10^{(0.108*T-1.3*pH)}$ describes the relationship between pH, temperature (T), and the rate of denaturation (k) where k_0 represents a denaturation constant of a protein (Offer and Knight, 1988). This

equation indicates that with increasing temperature and decreasing pH the rate at which protein is denatured increases.

Myoglobin, as previously mentioned, is a protein and is responsible for imparting the characteristic red or pink color to pork. As the myoglobin is denatured, it loses its ability to bind cations in a manner that produces the color hues desirable in pork (Lawrie, 1985). At pH below 6.1, the reflectance of pork begins to be adversely impacted (Warriss and Brown, 1987). If the pH is maintained at higher levels for longer periods of time, the amount of protein denatured in that time would be lower (offer and Knight, 1988). This decreased amount of protein denatured would result in more myoglobin in its native form (Offer and Knight, 1988; Murray et al., 1996). These results could lead to better redness of pork.

The myosin head shrinks when it is denatured, resulting in an increase in water loss because of sarcomeric shrinkage (Offer, 1991). The shrinkage is implicated as a major cause of increased water loss under adverse environmental conditions. In the PSE condition, the amount of myosin denatured is increased compared with normal pork, but in the RSE condition, the myosin denaturation is similar to normal meat (Warner et al., 1996). The RSE meat had higher myosin denaturization than did dry firm and dark (DFD) meat (Warner et al., 1996). These results implicate a high degree of sensitivity between the amount of myosin denatured and the water holding capacity of pork.

The glycogen phosphorylase was also more bound to the sarcoplasmic proteins than in RSE meat than in normal meat indicating a low pH and high temperature environment in the pre-rigor meat (Warner et al., 1996). Because the pH and temperature conditions were not optimum prior to chilling, the carcass had increased drip loss but the color was not

different from normal pork (Warner et al.,1996; Offer and Knight, 1988). The interaction between rate of pH decline and time after slaughter, and the relation between pH and drip loss, have been studied extensively (Warriss, 1982; Lesiak et al., 1996; Enfalt et al., 1993; Barbut and Mittal, 1993; Warriss and Brown, 1987; Kauffman et al., 1993; McCaw et al., 1997; van der Wal, 1997). These research studies present evidence that the ultimate pH, as well as the rate of pH decline, can have an impact on the drip loss of a pork product.

Although higher drip loss is observed in both cases, the method by which ultimate pH and the rate of pH decline affect the drip loss may be different. The effect of the ultimate pH on water loss is related to the Napole gene. The increased glycogen stored in pigs with the Napole gene results in pork with low ultimate pH and high water loss (Monin and Sellier, 1985). The high drip loss associated with the Napole gene is most likely because of the proximity of the pH of this meat to the isoelectric point. The pH could also be low enough that the protein is denatured even at low temperature (Offer and Knight, 1988). The rate of pH decline could be acting through protein denaturation or by altering the binding of phosphorylase (Warner et al., 1996; Offer and Knight, 1988). The equation $k_0 10^{(0.108*T-1.3*pH)}$ described previously indicates that temperature and pH can off set the effects of one another on rate of protein denatured. This relationship also allows for the rate of pH decline to be important for the rate of protein denaturization. After the animal is exsanguinated, the temperature of the carcass is relatively high. If the pH were to remain higher until the carcass were chilled, less protein would be denaturization.

Temperature

Temperature in the postmortem animal initially rises, and this rise is greater in animals with increased glycolytic rates (van der Wal et al., 1993; Hedrick et al., 1994). This elevation in body temperature is due to loss of homeostatic mechanism post-mortem. During life, heat is dissipated by blood flow near the skin surface and by evaporation because of respiration (Ganong, 1995). These mechanisms of dissipating heat are not functional in the post-mortem carcass. Heat is produced post-mortem from the chemical reactions involving the generation and hydrolysis of ATP (Hedrick et al., 1994). The combination of heat production and the loss in ability to dissipate heat results in temperature rises of 0.2 to 3°C upon exsanguination and remains elevated until the carcass is eviscerated (van der Wal et al., 1993; Hedrick et al., 1994). The temperature affects the enzymatic activities and protein denaturation during the conversion of muscle into meat.

Enzymes have an optimal temperature range just as they have an optimal pH range (Murray et al., 1996). The activity of enzymes may or may not be beneficial in terms of their effects on meat quality. The activity of the calpain system has a positive effect on the tenderness of meat (Huff-Lonergan et al., 1996). However, inhibition of certain enzyme activity may enhance meat quality for certain other characteristics. Inhibition of glycolytic enzymes would decrease the rate of pH decline that could have beneficial effects.

Proteins can also be denatured by increased temperature (Murray et al., 1996). This can be beneficial such as the case with cooking. At a constant pH, rises in temperature can increase the rate at which proteins are denatured as described by the equation $k_o 10^{(0.108*T-1.3*pH)}$ (Offer and Knight, 1988). This equation demonstrates that under pH or temperature extremes

the rate at which protein is denatured is independent of the other variable. The damaging effect of low pH can be mitigated by low temperature, and the damaging effect of high temperature can be mitigated by high pH (Offer and Knight, 1988). However, extremes of either low pH or high temperature cannot be mitigated.

Because the temperature of post-mortem muscle can have an impact on the quality characteristics of meat, modulation of post-mortem temperature can be effective at altering quality characteristics (Milligan et al., 1998). The chilling procedure has been examined to determine how to avoid negative aspects of temperature decline while taking advantage of possible benefits (Offer and Knight, 1988; Hedrick et al., 1994). Chilling causes the muscle temperature to decline rapidly and slows the rate of glycolysis (Newbold and Scopes, 1968). A decrease in the rate of glycolysis should aid in improving the quality of the meat produced.

Newbold and Scopes (1968) demonstrated that, as the temperature was lowered, the action of glycogen phosphorylase and phosphofructokinase limited the rate of glycolysis. Phosphorylase is the enzyme required for the liberation of glucose from glycogen (Murray et al., 1996). Phosphofructokinase activates fructose in the glycolytic pathway by adding a second phosphate to it (Murray et al., 1996). However, if the temperature is too low pre-rigor, a higher glycolytic rate is observed upon thawing (Newbold and Scopes, 1968). In the processing of beef carcasses, the muscle temperature at which the lowest glycolytic rate is achieved without inducing cold shortening is 10-12°C (Jeacocke, 1977). Poultry meat that is chilled too rapidly also undergoes cold shortening and results in higher shear values (Dunn et al., 1995). Cold shortening is not generally thought to be a problem in pork; however, slow

chilled pork may be more tender than that which has been blast chilled (Møller and Kirkegaard, 1987).

If chilling is inadequate or delayed, the resulting pork will have an increased incidence of PSE regardless of the genotype of the pig (Honkavaara, 1989). Delayed chilling of poultry carcasses has also shown that the pH decline continued more rapidly and water loss increased with increasing time prior to chilling (Lesiak et al., 1996). Chilling is, therefore, an important step for quality considerations when processing animals. Quick chilling has been used to improve quality (Hedrick et al., 1994). The color and water holding are improved with quicker chilling after slaughter (Hedrick et al., 1994).

Modulation of Glycolysis

The glycolytic rate in the post-mortem animal is responsible for the pH decline. Any alteration to the rate at which glycolysis occurs will consequently alter the pH decline. As was discussed, the temperature can affect the rate at which glucose is converted to lactic acid. The enzymes involved in the glycolytic pathway may have their activities altered by the application of exogenous compounds. Sodium bicarbonate fed shortly prior to slaughter did slow the glycolytic rate; however, this treatment did not impact the subsequent meat quality (Ahn et al., 1992). Analysis of blood indicated that maximal response to the treatment occurred too early pre-mortem to have impact post-mortem.

In other research, dantrolene, a drug used to treat malignant hypothermia, has been used to lower the glycolytic rate (Klont et al., 1994). The treatments were applied to pigs that were carriers for the halothane gene and to normal pigs. The decrease in glycolytic rate induced by the drug treatment had a positive effect on the drip loss in both populations of

animals. Enzymatic inhibition of glycolysis may be an effective method to minimize drip loss without adversely affecting the tenderness of pork.

Orally Active Glycolytic Inhibitors

Inhibition of glycolysis to improve the quality of pork has the potential to be valuable to the pork industry. For a treatment to be effective, it needs to inhibit one of the enzymes in the glycolytic pathway. It must retain this activity in the post-mortem animal. The treatment should be applied in a manner so as to reduce possible detriment to the safety of the product or the people processing the animal. Instead of using an injection procedure with contamination and worker safety risks, the orally active compound would present minimal risks. However, with an orally active compound, the safety for the consumer must be addressed. The meat needs to have a concentration of residue that would be in a safe range for consumers. The compound would have to be digestible so as to minimize the amount to be ingested. The compound would also need to have the potential to be transported in the animal to the muscle.

Oxalate

Oxalate has been shown to inhibit pyruvate kinase (Reed and Morgan, 1974). Pyruvate kinase is the enzyme required for conversion of phosphoenolpyruvate (PEP) to pyruvate (Murray et al., 1996). This is an exergonic reaction and thus produces ATP from ADP (Murray et al., 1996). Reed and Morgan (1974) found that oxalate competitively inhibits pyruvate kinase by interfering with the binding of PEP. The inhibition constant (K_i), the amount of oxalate required for reduction of the activity of the enzyme 50%, is 6 μM (Reed and Morgan, 1974). For the enzyme inhibition to be effective, the muscle enzyme

must be inhibited but the red blood cell must not for animal viability. Red blood cells obtain all of their energy via glycolysis (Murray et al., 1996). If the red blood cells cannot obtain ATP, they will lyse. The K_i of liver (2.8 mM) and erythrocyte (.27 mM) pyruvate kinases are higher than muscle pyruvate kinase (Buc *et al.*, 1978).

Oxalate is a naturally occurring compound. It is found in relatively high quantities in foods such as rhubarb, peanuts, and chocolate (Kasidas and Rose, 1980). It is known that oxalate can be absorbed (Hanes et al., 1999; Holmes et al., 1995; Prenen et al., 1984). It is, therefore, possible that oxalate could effectively be absorbed and inhibit glycolysis in the animal. These facts make it an option for studying the effects of a glycolytic inhibitor on meat quality. Because oxalate does occur in foods at a level similar to or lower than the amount that would be required to be effective to inhibit glycolysis, it would pose a limited risk to the consumer. Some people are prone to kidney stones, and they avoid oxalate-containing foods. Addition of oxalate to pig diets could affect their decision whether to purchase pork from treated animals.

Quercetin

Another compound that is an effective glycolytic inhibitor is quercetin. Quercetin is a flavonoid. Quercetin is a conjugated ring structure that occurs naturally in plant sources (Lister et al., 1994). Quercetin is an inhibitor of lactate dehydrogenase activity with a K_i of 1 μM (Grisiola et al., 1975). At 5 μM, lactate dehydrogenase is completely inhibited *in vitro* (Grisiola et al., 1975). Little is known of the mechanism of this inhibition. However, based on the structure of quercetin, it is most likely that this compound disrupts the ability of NADH to bind with the enzyme for potentiation of this reaction.

There is evidence that quercetin is absorbed (10 to 50 %) when taken orally in humans (Hollman et al., 1995; Manach et al., 1995; Conquer et al., 1998). It is therefore possible for the compound to be absorbed and inhibit glycolysis. Quercetin is a naturally occurring compound in plants such as apples, onions, and grapes (Manach et al., 1995). This compound has also been studied for possible benefits in preventing heart disease (Conquer et al., 1998). This compound is not only safe but may have health benefits to the consumer.

Vitamin C

Another compound that could be used to improve pork quality via glycolytic inhibition is vitamin C. Vitamin C would not directly inhibit glycolysis. However, the vitamin C is converted (5 %) to oxalate in animals when it is provided in excess of the amount required for antioxidant functions (Englard and Seifer, 1986). The oxalate could then inhibit pyruvate kinase as described previously. Vitamin C would be absorbed and would therefore have the potential to be an effective technology.

Zinc

One other compound that inhibits glycolysis is zinc (Ikeda et al., 1980). The amount of zinc required for the K_i level to be achieved is 10 μM if there are no chelating compounds present, but 200 μM and 18 μM in the presence of 10 mM of either histidine or carnosine, respectively (Ikeda et al., 1980). These researchers determined that the inhibition of glycolysis was because of inhibition of phosphofructokinase. This inhibition is most likely due to interference with the divalent ion balance required for ATP synthesis. Supplemental zinc is absorbed and would therefore be available to the muscle (Hill et al., 1987).

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CHAPTER 3. EFFECT OF SODIUM OXALATE ON THE QUALITY OF PORK

A paper to be submitted to the *Journal of Animal Science*

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Abstract

Nine sets of three littermate barrows from a high lean, halothane negative strain were used in Experiment 1 to determine the effects of dietary sodium oxalate, an inhibitor of pyruvate kinase, on the postmortem biochemistry of pig muscle, and in turn on pork quality. At BW of 111 ± 6.8 kg, pigs were offered a basal diet (5 g/kg BW) containing 0, 1.98 and 9.69% sodium oxalate for 4 h pre-transport which was estimated to provide body water concentrations of oxalate equivalent to 0, 2, and 10 times the K_i of pyruvate kinase. Pigs were transported to the ISU Meat Laboratory, stunned, exsanguinated, and scalded. Muscle temperature and pH were determined in the longissimus and semimembranosus muscles in each carcass side at 22, 45, 90, and 180 min postmortem. At 45 min post mortem, the right carcass side was quick chilled at 0°C while the left side was slow chilled at 18.3°C through 180 min. postmortem and then at 0°C. At 24 h postmortem, chops (2.5 cm) were taken from each muscle and side for measurement of percentage water loss (WL) and color on days 0, 3, 6, 9, and 12 of retail storage (5.6°C). Pigs consumed 342 g, 360 g and 100 g of feed resulting in intakes of 0, 7.0, and 9.7 g of sodium oxalate. Dietary sodium oxalate additions pooled across time postmortem increased muscle pH (5.90, 5.99, and 5.99; $P < 0.06$) independent of chilling temperature and muscle type. Dietary sodium oxalate also reduced pork WL (11.4,

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and 10.1, 9.6%, $P < .05$). Dietary sodium oxalate did not affect Hunter L* or Hunter a* color scores. In Experiment 2, barrows at a BW of 29.9 ± 3.6 kg were offered a basal diet (15g/kg BW) containing 0 or .69% sodium oxalate, estimated to be equivalent to 0 and 2 times K_i, for 4 h prior to transport. Pigs were killed and processed as in Experiment 1 except that the pigs were not scalded and both carcass sides were slow chilled at 18.3°C through 180 min postmortem. The dietary sodium oxalate increased muscle pH (6.18 vs. 6.22; $P < .05$) and minimized WL (5.99 vs. 5.27%, $P < .07$) pooled across time postmortem but did not alter pork color scores. Based on these data, short term feeding of sodium oxalate premortem is an effective technology for slowing postmortem pH decline and minimizing pork WL during storage.

Introduction

The quality attributes of water holding capacity and color in pork products are significant concerns to the pork industry. Loss of water from pork products results in less saleable product weight, impaired processing characteristics, and lower consumer appeal (NPPC, 1994). A pale color in pork products also impairs export opportunities. These problems are caused, in part by the continuation of glycolysis in muscle postmortem that results in the accumulation of lactic acid before the muscle is chilled. Muscle temperature postmortem initially increases and then declines slowly until the carcass is chilled. High muscle temperature combined with declining muscle pH results in an environment conducive to protein denaturization (Offer and Knight, 1988). Greater rates of protein denaturization are related to increases in water loss from meat (Offer and Knight, 1988). A slower pH decline in muscle postmortem is associated with a lower rate of protein denaturization and greater water holding in meat (Penny, 1969).

Inhibition of a glycolytic enzyme would decrease the rate at which lactic acid is formed. Oxalate concentration required to inhibit muscle pyruvate kinase to half its normal activity (K_i) is 6 μM which is lower than the concentration which inhibits liver (2.8 mM) or erythrocyte (.27 mM) pyruvate kinase (Reed and Morgan, 1974; Buc et al., 1978). Oxalate associates with pyruvate kinase in a manner that results in a competitive inhibition of the enzyme (Reed and Morgan, 1974). Oxalate is a natural component of foods such as spinach, rhubarb, and tea (Kasidas and Rose, 1980). Oxalate is absorbed either as oxalate or as a calcium salt (Farooqui et al., 1983; Hanes et al., 1999). The objective of this study was to determine the effect of short term feeding of oxalate, a glycolytic inhibitor, on the postmortem pH decline in pig muscle and, in turn, pork quality.

Material and Methods

Experiment 1

Nine sets of three littermate barrows from a lean, halothane-negative genetic strain were used. Pigs were individually penned on slotted floors in .61 m x 3.1 m pens in a thermal neutral environment maintained at 21 to 24°C. Before the experiment started, pigs were allowed to consume, ad libitum, a basal diet consisting of corn and soybean meal mix fortified with minerals and vitamins (Table 1). When pigs reached a BW of 111 ± 7 kg, three pigs within each litter were randomly assigned to one of three dietary treatments consisting of the basal diet (.10% sodium) supplemented with 0, 1.98, or 9.69% sodium oxalate. These amounts of sodium oxalate were estimated to provide concentrations of oxalate in the body water equivalent to 0, 2, and 10 times the inhibitory constant (K_i) of oxalate for muscle pyruvate kinase (Reed and Morgan 1974). These concentrations are based on the following assumptions: body water content of 60%, absorption of 1% of oxalate from sodium oxalate

(Heaney and Weaver 1989), and complete equilibration of water soluble oxalate in the body water. The sodium oxalate (99.9% purity) was obtained from Sigma Chemical (P.O. Box 14508, St. Louis, MO 63178). To enhance the consumption of the allocated feed (5 g/kg BW for 4 h before transport), pigs were removed from feed for 4 h (2300 to 0300 h) and then allowed ad libitum access to the allocated experimental feed for 4 h (0300 to 0700 h). Pigs were allowed ad libitum access to water throughout.

Pigs were loaded, transported (5 km) to the Iowa State University Meat Laboratory, and then killed in littermate groups within 60 min of arrival. Pigs were not provided access to feed or water while in lairage at the Meat Laboratory. Pigs were stunned for 7 s with 280 v, laid on their side to reduce catecholamine release (Troeger and Woltersdorf, 1989), and killed by exsanguination within 7 s of stunning. Pigs were allowed to bleed for 5 min and then placed in a scalder-dehairer (64°C) at 7 min post-stun where the hair was mechanically removed with rubber paddles. Pigs were removed from the scalder, singed, rinsed, eviscerated, and split into two carcass sides at 15, 16, 18, 20, and 25 min postmortem, respectively.

At 45 min postmortem, the right carcass side was quick chilled at 0°C, while the left side was slow chilled at 18.3°C through 180 min postmortem and then at 0°C. These conditions simulated quick and slow chilling environments, respectively (Landes et al., 1971; Kauffman et al., 1993). Muscle pH and temperature were determined in the longissimus (LM) and semimembranosus (SM) muscles at 22, 45, 90, 180 min postmortem. At 24 h postmortem, an ultimate pH was determined. The LM and SM muscles were then cut into 2.5 cm thick chops beginning at the 10^{th} rib and 2.5 and 5.0 cm posterior to the anterior head, respectively. The chops were trimmed of subcutaneous fat and bone and stored on styrofoam

trays with absorbent pads under oxygen permeable wrap at 5.6°C for 12 d. The chops were placed on racks in a cooler with overhead fluorescent lights on for 24 h each day.

Muscle pH and temperature were measured in the LM and SM muscles from both chilling environments by using an ISFET pH probe (Sentron 3001-002; div. of Medtronic Synetics; 850 Victoria Street N, MS v215; Shoreview, MN 55126) and thermister. The pH probe and thermister are accurate to .02 pH units and .10°C, respectively. Muscle pH and temperature measurements were made in the LM muscle at a point posterior to the third, fourth, fifth, and sixth rib at 22, 45, 90, and 180 min postmortem, respectively. The SM muscle measurements were made about 1.5, 2.5, 3.5, and 4.5 cm from the posterior head of the muscle, respectively. Pork WL and color scores were determined on day 0, 3, 6, 9, and 12 d of storage. Pork WL was determined by unwrapping and weighing the same chop to the nearest milligram on each designated day of storage. After weighing, the chop was placed on a new absorbent pad and rewrapped in oxygen permeable over-wrap. Pork color was determined in a second chop by using the Hunter L*a*b* on a model JB-1201 M(A) Hunter LABSCAN machine (Hunter Assoc. Laboratory, Inc.; 11495 Sunset Hills road; Reston, VA 22090). Pork cooking loss and tenderness were determined on samples on d 12 of storage. Cooking loss was determined by heating a pre-weighed chop in a Farberware convection oven (Syrtech corp., 175 McClellan Hwy., East Boston, MA 02128) to an internal temperature of 68°C and then reweighing the chop. Pork tenderness was estimated on a raw, 2.5 cm chop via the Star Probe technique by using the Instron Universal Testing Machine series 4500 (Instron Corp.; 100 Royall Street; Canton, MA 02021).

Chops from the right LM muscle from three pigs, one from each dietary treatment, were analyzed for oxalate content. Three subsamples weighing 2 g were obtained from the

right LM muscle from each of three pigs. The 2 g sub-samples were macerated in 3 ml of 2 N HCl using a polytron (Brinkman; Cantiague Road; Westbury, NY 11590). Samples were centrifuged at 3020 G. The supernatant was frozen and subsequently analyzed by capillary electrophoresis (Holmes et al., 1995) by Ross Holmes (Dept. of Urology, Wake Forest School of Medicine, The Bowman Gray Campus, Winston Salem, NC 27157). The assay allows an oxalate recovery of 92% with a coefficient of variation of 3.3% (Holmes et al., 1995). Holmes et al.(1995) was able to detect .4 mg oxalate/100 g in orange juice by using this assay.

Data were analyzed by analyses of variance techniques by using the GLM and MIXED procedures of SAS (SAS Institute, 1998). Data were analyzed as a split plot design with dietary sodium oxalate considered the whole plot treatment, chilling environment as the split plot treatment, and muscle as the split-split plot. Orthogonal contrasts were used to analyze the effects of dietary sodium oxalate concentration. Responses over time were analyzed as repeated measures. The error terms for testing dietary sodium oxalate (DO), chill environment (CE), time postmortem (TM), and day of storage (DS) were: DO x replicate (REP), CE x REP, TM x REP, and DS x REP, respectively. The error terms for testing DO x CE, DO x TM, DO x DS, CE x TM, and CE x DS were DO x CE x REP, DO x TM x REP, DO x DS x REP, CE x TM x REP, and CE x DS x REP, respectively. The error terms used to test for DO x CE x TM and DO x CE x DS were DO x CE x TM x REP and DO x CE x DS x REP. Pork water loss was adjusted for difference in initial chop weight. All means reported are least square means.

Experiment 2

Fourteen sets of two barrows from a lean, halothane negative genetic strain were used. Pigs weighing 29.7 ± 3.6 kg were offered (15g/kg BW) a basal diet (.23% sodium, Table 2) containing 0 or .69% sodium oxalate during the 4 h period before transport to the Iowa State University Meat Laboratory. These dietary sodium oxalate inclusions were estimated to provide oxalate body water concentrations equivalent to 0 and 2 times the K_i for muscle pyruvate kinase. The pigs were housed in .61 m X 1.22 m pens in a thermal neutral environment (26.7°C). The procedures employed for feeding, transporting, killing, processing, and sampling were the same as for experiment one with the following exceptions. Pigs were not scalded or mechanically dehaired. Both carcass sides were slow chilled at 18.3°C through 180 min postmortem and then at 0°C. Muscle pH and temperature were determined only in the LM muscle because of the inadequate size of the SM muscle. Measurement of the pork cooking loss, tenderness (Star probe), and oxalate concentration were not taken.

Pigs were randomly allotted to dietary treatment from outcome groups based on BW. Data were analyzed as a randomized complete block design by using analysis of variance techniques (SAS, GLM Procedure of SAS, 1998). Responses over time were analyzed as a repeated measure. Least square means are reported.

Results

Experiment 1

The mean BW of pigs in the 0, 2X, and 10X treatment groups were 109.1, 113.5, and 111.5 kg resulting in feed allocation of 545, 568, and 557 g, respectively (Table 3). During the 4 h pre-transport feeding period, pigs consumed 342, 360, and 100 g of the allocated feed in the 0, 2X, and 10X treatment groups, resulting in oxalate intakes of 0, 7.0, and 9.7 g,

respectively (Table 3). These intakes are estimated to provide oxalate concentrations in body water equivalent to 0, 1.28, and 1.80 times the K_i for muscle pyruvate kinase.

Dietary sodium oxalate, chill temperature, and time postmortem each influenced postmortem muscle biochemistry (Table 4). As expected, the quick chill environment resulted in a lower ($P < .01$) muscle temperature, slower ($P < .01$) postmortem muscle pH decline, and a greater ($P < .05$) ultimate pH.

Dietary sodium oxalate ingestion resulted in greater ($P < .06$) postmortem muscle pH (22 to 180 min postmortem) independent of chill temperature, muscle type, and time postmortem. Specifically, sodium oxalate consumption, pooled across time postmortem, increased muscle pH in the quick chilled (6.00, 6.08, and 6.07) and in slow chilled side (5.79, 5.89, and 5.91; Table 4). The magnitude of the response was similar for the 2X and 10X levels of dietary inclusion. Dietary sodium oxalate did not alter muscle temperature in the quick chilled (30.8, 30.5, and 30.5°C) or the slow chilled environment (31.9, 31.9, and 31.6°C). Ultimate muscle pH (24 h) was not altered by dietary sodium oxalate in either the quick chill (5.57, 5.57, and 5.65) or slow chill environment (5.51, 5.54, and 5.60, Table 4).

Dietary sodium oxalate, chill temperature, and days of storage also influenced pork quality traits (Table 5). The quick chill environment resulted in lower ($P < .01$) WL. The quick chill environment also resulted in lower ($P < .10$) Hunter L* scores but did not alter the Hunter a* color scores. Chill environment also did not alter pork cooking loss or penetration resistance, which is a measure of pork tenderness.

Dietary sodium oxalate consumption, pooled across storage time, lowered ($P < .05$) pork WL by 1.2 to 1.9 percentage units in the quick and slow chill environments. The magnitude of response was similar for the 2X and 10X treatment groups. The magnitude of

the response over days of storage however, was different for the two chill environments. Dietary sodium oxalate lowered WL in the quick chill environment by a relatively constant amount (1.25, 1.15, 1.20, and 1.20% on d 3, 6, 9, and 12, respectively). In contrast, dietary sodium oxalate lowered WL in the slow chill environment by progressively larger amounts as storage progressed (1.55, 1.90, 2.00, and 2.10% on d 3, 6, 9, and 12, respectively). Consumption of sodium oxalate did not alter pork cooking loss (29.5, 29.6, and 28.4%) or penetration resistance (3.00, 2.97, and 3.00 kg) in chops after 12 d of storage (Table 6). Oxalate was not detected in the three meat samples analyzed.

Experiment 2

The pig BW, feed allocation, actual feed intake, and oxalate intakes are reported in Table 7. Voluntary feed intakes were similar among the 0 and 2X treatment groups (325 and 383 g/pig) which is in agreement with the results of Experiment 1 (Table 3 and Table 7). Pigs from Experiment 2 consumed quantities of oxalate estimated to provide body water concentration (0 and 1.68 K_i) intermediate in terms of K_i to that of pigs in Experiment 1 (1.28 and 1.80 K_i) (Table 3 and Table 7). As in Experiment 1, sodium oxalate consumption resulted in greater postmortem muscle pH with the greatest responses occurring at 180 min postmortem (Table 8). Muscle temperature postmortem was not altered by dietary sodium oxalate. The slower pH decline induced by sodium oxalate was associated with a lower percentage pork WL during storage (Table 9). Again in this experiment, dietary sodium oxalate treatment did not alter the color scores of these carcasses as measured by Hunter L* and Hunter a* values (Table 9).

Discussion

The differences in muscle pH in this study were expected because oxalate is an inhibitor of pyruvate kinase (Reed and Morgan, 1974; Buc et al., 1978). The inhibition of pyruvate kinase would decrease the amount of lactate formed, therefore reducing the rate of pH decline in muscle postmortem. By increasing the muscle pH, the rate at which protein is denatured is decreased (Offer and Knight, 1988). This biochemical change normally results in pork with a greater ability to hold water and thus with less purge (Offer and Knight, 1988). The WL and color scores of pork are not linked as closely as once thought. Evidence of this linkage can be deduced from the occurrence of red, soft, and exudative (RSE) pork (Warner et al., 1997). This class of pork has poorer water holding characteristics but has normal color. The high WL that occurs in these products in the presence of normal color indicates that WL may be more sensitive to temperature and/or pH than color.

The fact that oxalate additions decreased the initial rate of pH decline and improved WL at both chill temperatures and across the entire time observed indicates that this technology is effective under a wide range of environmental conditions. Dietary sodium oxalate did not completely overcome the WL increase associated with slow chilling, but it improved the WL value above that of the quick chill carcasses from pigs fed the basal diet. The dietary sodium oxalate treatment would help decrease the variability in pork products and would improve the overall quality associated with those products as measured by WL.

The ingestion of 61.7 to 87.0 mg sodium oxalate/kg BW in Experiment 1 resulted in equivalent reductions in initial rate of pH decline and subsequently in pork WL during storage. The lower feed intake of the pigs in the 10X treatment group could have influenced

pork quality in a positive manner (Offer and Knight, 1988). The pigs in the 10X treatment group had higher sodium oxalate intakes than did those in the 2X treatment group.

The results of the second experiment using 30 kg pigs confirmed the results of the first experiment with 111 kg pigs. The lower pork WL and greater Hunter color scores in the second experiment were because of the greater number of red muscle fibers compared to white muscle fibers in smaller pigs as compared with the larger pigs typical during growth and development (Hedrick et al., 1994). However, based on these data, a smaller pig can be used as a model to evaluate the efficacy of orally active glycolytic inhibitors on pork quality. The smaller pigs consumed 86.4 mg sodium oxalate/kg BW that closely approximated that of the larger pigs in the 10X group in experiment one. Sodium oxalate addition lowered postmortem muscle pH at 90 to 180 min postmortem by .13 to .07 in the 111 kg pig and .05 to .19 units in the 30 kg pig. These shifts in pH decline resulted in a 12 percent reduction in WL in both experiments.

Implications

Oxalate is a naturally occurring compound that inhibits glycolysis. Short term feeding of oxalate provided as sodium oxalate is an effective technology for improving the water holding capacity of pork products.

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Table 1. ***Experiment 1.*** **Basal diet composition (%)**

Ingredient	% of Diet
Corn, yellow	82.445
Soybean meal, 48%	14.00
L-Lysine HCl	.20
Animal fat, stabilized	1.00
Dicalcium phosphate	.93
Limestone	.90
Salt	.25
TM-vitamin premix[a]	.25
Antimicrobial[b]	.025

[a] Contributed the following per kilogram of diet: vitamin A, 4405 IU; vitamin D_3, 1101 IU; vitamin E, 22 IU; niacin 33 mg; pantothenic acid, 17.6 mg; riboflavin, 6.6 mg; vitamin B_{12}, 22 μg; Fe, 88 mg; Zn, 75 mg; Cu, 8.8 mg; Mn, 30 mg; I, 1 mg.

[b] Contributed the following per kilogram of diet: bacitracin methylene disalicylate, 2.0 mg.

Table 2. ***Experiment 2.*** **Basal diet composition (%)**

Ingredient	% of Diet
Corn, yellow	50.56
Whey, dried	10.00
Soybean meal, 48%	33.50
L-Lysine HCl	.20
DL Methionine	.10
Animal fat, stabilized	1.00
Dicalcium phosphate	2.20
Limestone	.78
Salt	.25
TM-vitamin premix[a]	.41
Antimicrobial[b]	1.00

[a] Contributed the following per kilogram of diet: vitamin A, 6608 IU; vitamin D_3, 1652 IU; vitamin E, 33 IU; niacin 50.7 mg; pantothenic acid, 26.4 mg; riboflavin, 9.9 mg; vitamin B_{12}, 33 μg; Fe, 105 mg; Zn, 90 mg; Cu, 10.5 mg; Mn, 36 mg; I, 1.2 mg; Se, .3 mg.

[b] Contributed the following per kilogram of diet: carbadox, 55 mg.

Table 3. ***Experiment 1.*** **Pig BW and feed intakes during the 4 h pre-transport period.**

	Dietary sodium oxalate, K_i			
Criteria	**0**	**2X**	**10X**	**SEM**
Number of pigs	9	9	9	
Pig BW, kg	109.1	113.5	111.5	1.50
Feed allocation, g/pig	545	568	557	8
Feed intake, g/pig[a]	342	360	100	72
Oxalate intake, g/pig	0	7.0	9.7	5.8

[a] Effect of oxalate 2X vs. 10X, $P < .01$.

Table 4. ***Experiment 1.*** **Effect of dietary sodium oxalate on muscle pH and temperature postmortem (data pooled across muscle).**

Criteria	Chill temperature	Time post-mortem	Dietary sodium oxalate, K_i 0	2X	10X	SEM[a]
pH[b]	Quick	22 min	6.36	6.40	6.40	0.12
		45 min	6.28	6.21	6.29	
		90 min	5.97	6.12	6.10	
		180 min	5.70	5.81	5.77	
		Mean	6.00	6.08	6.07	
	Slow	22 min	6.34	6.43	6.43	
		45 min	6.18	6.22	6.27	
		90 min	5.67	5.82	5.86	
		180 min	5.50	5.59	5.57	
		Mean	5.79	5.89	5.91	
Ultimate pH[c]	Quick	24 h	5.57	5.57	5.65	0.12
	Slow	24 h	5.51	5.54	5.60	
Temperature, °C[d]	Quick	22 min	35.9	35.7	35.8	0.04
		45 min	35.4	34.8	35.5	
		90 min	29.5	29.5	28.8	
		180 min	22.4	22.5	21.9	
		Mean	30.8	30.5	30.5	
	Slow	22 min	34.7	34.7	34.9	
		45 min	34.8	34.7	34.5	
		90 min	31.8	31.9	30.8	
		180 min	26.3	26.8	26.0	
		Mean	31.9	31.9	31.6	

[a] The SEM is calculated from the oxalate x chill temperature x muscle x period x replicate term.

[b] The pH was analyzed and means determined as hydrogen ion concentration and were converted for presentation, effect of oxalate 0 vs. 2X & 10X, $P < .06$; effect of chill temperature, $P < .01$; quadratic effect of time, $P < .01$; chill temperature x time postmortem, $P < .01$.

[c] Effect of chill temperature, $P < .05$.

[d] Effect of chill temperature, P < .01; quadratic effect of time, P < .01; effect of chill temperature x time postmortem, P < .01.

Table 5. ***Experiment 1.*** **The effect of dietary sodium oxalate on pork water loss (WL) and color scores during storage (data pooled across muscle).**

Criteria	Chill temperature	Time day of storage	Dietary sodium oxalate, K_i 0	2X	10X	SEM[a]
WL, %[b]	Quick	0 to3	7.1	6.1	5.6	0.03
		3 to 6	9.5	8.3	8.4	
		6 to 9	11.1	10.0	9.8	
		9 to 12	12.1	11.0	10.8	
		Mean	9.9	8.9	8.5	
	Slow	0 to 3	9.7	8.3	8.0	
		3 to 6	12.3	10.7	10.1	
		6 to 9	13.9	12.3	11.5	
		9 to 12	15.0	13.3	12.5	
		Mean	12.8	11.3	10.6	
Hunter L*[c]	Quick	0	49.6	48.8	47.7	0.15
		3	50.6	53.0	52.3	
		6	51.8	54.0	54.0	
		9	53.6	54.5	54.4	
		12	54.4	54.7	54.9	
		Mean	52.0	53.0	52.6	
	Slow	0	50.8	50.5	50.5	
		3	53.7	51.9	52.7	
		6	53.8	52.9	52.5	
		9	54.8	53.9	53.7	
		12	55.7	55.5	54.5	
		Mean	53.8	52.9	52.8	
Hunter a*	Quick	0	5.5	5.3	5.9	0.04
		3	6.8	6.4	6.2	
		6	6.1	5.6	5.5	
		9	5.1	5.2	5.0	
		12	4.2	4.3	3.9	
		Mean	5.5	5.3	5.3	

Table 5. Continued

Slow	0	6.3	5.8	5.3
	3	6.8	6.5	6.4
	6	5.8	5.7	5.8
	9	5.1	5.1	5.0
	12	4.4	4.7	4.0
	Mean	5.7	5.6	5.3

[a] The SEM is calculated from the oxalate x chill temperature x muscle x period x replicate term.

[b] Effect of oxalate 0 vs. 2X & 10X, $P < .05$; effect of chill temperature, $P < .01$; quadratic effect of days, $P < .01$; effect of dietary sodium oxalate x chill temperature x days of storage, $P < .06$.

[c] Effect of chill temperature, $P < .10$; quadratic effect days, $P < .01$; effect of dietary sodium oxalate x days of storage, $P < .05$; effect of chill temperature x days of storage, $P < .06$.

Table 6. ***Experiment 1.*** **Effect of dietary sodium oxalate on pork cooking loss and penetration resistance (data pooled across muscle type)**

		Dietary sodium oxalate, K_i			
Criteria	**Chill temperature**	**0**	**2X**	**10X**	**SEM**
Cooking loss (%)	Quick	29.4	29.5	27.8	.20
	Slow	29.6	29.6	29.1	
Penetration resistance (kg)	Quick	3.04	2.96	2.95	.04
	Slow	2.92	2.99	3.05	

Table 7. ***Experiment 2.*** **Pig BW and feed intake during the 4 h pre-transport period.**

Criteria	Dietary sodium oxalate, K_i 0	2X	SEM
Number of pig	14	14	
Pig BW, kg	29.6	30.1	.48
Feed allocation, g/pig	448	455	6
Feed intake, g/pig	325	383	9
Oxalate intake, g/pig	0	2.6	.26

Table 8. ***Experiment 2.*** **Effect of dietary sodium oxalate on muscle pH and temperature postmortem (longissimus muscle only)**

Criteria	Time Post mortem	Dietary sodium oxalate, K_i 0	2X	SEM
pH[a]	22 min	6.45	6.38	.02
	45 min	6.24	6.25	
	90 min	6.12	6.17	
	180 min	5.89	6.08	
	Mean	6.18	6.22	
Temperature, °C[b]	22 min	32.8	32.8	.05
	45 min	30.5	30.6	
	90 min	28.3	28.3	
	180 min	19.5	19.6	
	Mean	27.8	27.8	

[a] The pH was analyzed and means determined as hydrogen ion concentration and were converted for presentation, effect of oxalate, $P < .05$; linear effect of time $P < .01$; effect of dietary sodium oxalate x time postmortem, $P < .01$.

[b] quadratic effect of time, $P < .01$.

Table 9. ***Experiment 2.*** **Effect of dietary sodium oxalate on pork water loss (WL) and color scores during storage. (data pooled across muscle)**

Criteria	Day of storage	Dietary sodium oxalate, K_i 0	2X	SEM[a]
WL, %[b]	0 to 3	2.9	2.4	.07
	3 to 6	5.3	4.6	
	6 to 9	7.1	6.3	
	9 to 12	8.6	7.8	
	Mean	6.0	5.3	
Hunter L*[c]	0	45.8	45.0	.08
	3	47.9	47.1	
	6	49.6	48.6	
	9	49.5	49.2	
	12	50.9	50.6	
	Mean	48.7	48.1	
Hunter a*[c]	0	6.8	7.1	.05
	3	8.9	9.2	
	6	8.8	9.1	
	9	8.4	8.3	
	12	7.3	7.2	
	Mean	8.1	8.2	

[a] The SEM is calculated from the oxalate x muscle x period x replicate term.

[b] Effect of oxalate, $P < .07$; quadratic effect of days, $P < .05$.

[c] quadratic effect of days, $P < .01$.

CHAPTER 4. EFFECT OF DIETARY QUERCETIN ON MEAT QUALITY OF PORK

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Abstract

Nine sets of three littermate barrows from a high lean, halothane negative genetic strain were used to determine the effects of dietary quercetin, an inhibitor of lactate dehydrogenase, on the postmortem biochemistry of pig muscle, and in turn, on pork quality. At BW of 108.7 ± 7.0 kg, pigs were offered a basal diet (5 g/ kg BW) containing 0, 2.5, and 12.4 ppm quercetin for 4 h pre-stunning which was estimated to provide body water concentrations of quercetin equivalent to 0, 2, and 10 times the K_i of lactate dehydrogenase. Pigs were transported 5 km to the ISU Meat Laboratory and killed within 60 min of arrival. Pigs were stunned, exsanguinated, and scalded. Muscle temperature and pH were determined in the longissimus and semimembranosus muscle on each carcass side at 22, 45, 90, and 180 min postmortem. At 45 min postmortem, the right carcass side was quick chilled at 0°C, whereas the left side was slow chilled at 18.3°C though 180 min postmortem and then 0°C. At 24 h postmortem, chops (2.5 cm) were taken from each of the two muscles of each side for measurement of water loss (WL) and color on d 0, 3, 6, 9, and 12 of storage. Chops were placed on absorbent pads in styrofoam trays, wrapped with oxygen permeable wrap, and stored at 5.6°C. Pigs consumed 342, 431, and 377 g of feed, respectively, resulting in intakes of 0, 1.07, and 4.68 mg of quercetin. Dietary quercetin additions, pooled across time

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postmortem, increased muscle pH (5.90, 5.97, and 6.00, $P < .10$) and the magnitude of change was greater in the slow chill environment. Dietary quercetin, pooled across day of storage, also decreased ($P < .11$) pork WL (11.4, 9.1, and 11.0%) independent of chill temperature and muscle type, but the response was greater ($P < .05$) for the 2X than for the 10X dose of quercetin. Dietary quercetin raised Hunter L* values in the quick chill but lowered them in the slow chill environment. Dietary quercetin addition lowered ($P < .01$) Hunter a* values 5.61, and 5.04, 4.99 for the three dosages. Based on these data, the premortem feeding of the dietary glycolytic inhibitor, quercetin, effectively slows postmortem muscle pH decline and minimizes WL in the subsequent pork product.

Introduction

The quality attributes of water holding capacity and color of pork products are a concern. Loss of water from pork products results in less saleable product weight, impaired processing characteristics, and lower consumer appeal (NPPC, 1994). A pale color in pork products also impairs export opportunities. These problems are caused, in part, by the continuation of glycolysis and the resulting accumulation of lactic acid in muscles postmortem. High muscle temperature combined with declining muscle pH results in an environment conducive to protein denaturization (Offer and Knight, 1988). Greater rates of protein denaturization are related to increases in the water loss in meat postmortem (Offer and Knight, 1988). Slower pH decline is associated with lower rates of protein denaturization and higher water holding in meat (Penny, 1969). Protein denaturization is negatively related to the subsequent water holding capacity and color of pork (Warner et al., 1997).

Inhibition of a glycolytic enzyme would decrease the rate at which lactic acid is formed. Feeding sodium oxalate, an inhibitor of pyruvate kinase, minimizes postmortem pH decline and WL from subsequent pork products (Kremer et al., 1999). Quercetin, a flavoniod, has been shown to inhibit muscle lactate dehydrogenase with a K_i of 1 μM in vitro (Grisiola et al., 1975). The exact nature of the inhibition has not been studied. At high concentration (15 μM), quercetin completely inhibits lactate dehydrogenase (Grisiola et al., 1975). Naturally abundant sources of quercetin include onions and apples (Manach et al., 1995). From an oral dose, 30% of quercetin is absorbed from the intestine and does not rapidly appear in the urine (Hollman et al., 1995).

The objective of this experiment was to determine the effect of short-term premortem feeding of quercetin, a lactate dehydrogenase inhibitor, on postmortem muscle pH decline and subsequent pork quality.

Material and Methods

Nine sets of three littermate barrows from a high lean, halothane-negative genetic strain were penned individually on slotted floors in .61 x 3.1 m pens in a thermal neutral environment maintained at 21 to 24 °C. Before the experiment started, pigs were allowed ad libitum access to a basal diet consisting of corn and soybean meal mix fortified with minerals and vitamins (Table 1). At 108.7 ± 7.0 kg BW, barrows were randomly allotted within litter to one of three dietary treatments consisting of the basal diet (5 g/kg BW) supplemented with 0, 2.5, or 12.4 ppm quercetin. These supplemental amounts of quercetin were estimated to provide concentrations of quercetin in body water equivalent to 0, 2, and 10 times the muscle K_i (1 μM) of quercetin for lactate dehydrogenase. These concentrations are based on the following assumptions: body water content of 60%, absorption of 30% for the quercetin

(Hollman et al., 1995), and complete equilibration of the quercetin in the body water. Quercetin fed in this study was, in a pure aglycone form, obtained from Sigma Chemical (P.O. Box 14508; St. Louis, MO 63178).

The pigs were transported, killed, and processed in the same manner as outlined by Kremer et al. (1999). At 45 min postmortem, the right carcass side was quick chilled at 0°C, while the left side was slow chilled at 18.3°C until 180 min postmortem and then at 0°C. These condition simulated quick and slow chilling environments, respectively (Landes et al., 1971; Kauffman et al., 1993). Muscle pH and temperature were determined in longissimus (LM) and semimembranosus (SM) muscles at 22, 45, 90, 180 min. At 24 h postmortem, ultimate pH of each muscle was determined. The LM and SM muscles were removed, cut into 2.5 cm thick chops beginning at the 10^{th} rib and 5.5 and 5.0 cm from the posterior from the anterior head of the SM muscle, respectively. The chops were trimmed of subcutaneous fat and bone, and stored on styrofoam trays with absorbent pads under oxygen permeable wrap at 5.6°C for 12 d. The chops were placed on racks in a cooler with overhead fluorescent lights on for 24 h each day.

Muscle pH and temperature were measured in the LM and SM muscles in both chilling environments by using an ISFET pH probe (Sentron 3001-002; div. of Medtronic Synetics; 850 Victoria Street N, MS v215; Shoreview, MN 55126) and thermister. The pH probe and thermister are accurate to .02 pH units and .10°C, respectively. Muscle pH and temperature measurements were made in the LM muscle posterior to the third, fourth, fifth, and sixth rib at 22, 45, 90, and 180 min postmortem, respectively. The SM muscle measurements were made about 1.5, 2.5, 3.5, and 4.5 cm from the posterior head of the muscle, respectively. Pork water loss (WL) and color scores were determined on d 0, 3, 6, 9,

and 12 d of storage. Pork WL was determined by unwrapping and weighing the same chop to the nearest mg on each designated day of storage. After weighing, the chop was placed on a new pad and rewrapped in oxygen permeable over wrap. Pork color was determined using the Hunter L*a*b* on a model JB-1201 M(A) Hunter LABSCAN machine (Hunter Assoc. Laboratory, Inc.; 11495 Sunset Hills road; Reston, VA 22090). Pork cooking loss and tenderness were determined on samples on d 12 of storage. Cooking loss was determined by heating each chop in a Farberware convection oven (Syrtech corp., 175 McClellan Hwy., East Boston, MA 02128) to an internal temperature of 68°C, then re-weighing the chop. Pork tenderness was estimated on a raw, 2.5 cm chop via the Star Probe technique using the Instron Universal Testing Machine series 4500 (Instron Corp.; 100 Royall Street; Canton, MA 02021).

The data were analyzed by analysis of variance technique by using the GLM and MIXED procedures of SAS (SAS, 1998). Data were analyzed as a split plot with dietary quercetin considered the whole plot treatment, chilling environment considered the split plot treatment, and muscle considered the split-split plot treatment. Initial chop weight was used as a covariate in the analysis of data for WL. Orthogonal contrasts were used to evaluate the effects of dietary quercetin concentration. Responses over time were analyzed as repeated measures. The pH was analyzed as the hydrogen ion concentration and means converted for presentation. The error terms for testing dietary quercetin (DQ), chill environment (CE), time postmortem (TM), and day of storage (DS) were: DQ x replicate (REP), CE x REP, TM x REP, and DS x REP, respectively. The error terms for testing DQ x CE, DO x TM, DQ x DS, CE x TM, and CE x DS were DQ x CE x REP, DQ x TM x REP, DQ x DS x REP, CE x

TM x REP, and CE x DS x REP, respectively. The error terms used to test for DQ x CE x TM and DQ x CE x DS were DQ x CE x TM x REP and DQ x CE x DS x REP.

The data from this experiment were pooled with data from a second glycolytic inhibitor (Kremer et al., 1999) for a backward stepwise multiple regression to determine the relative effects of muscle temperature, pH decline, and ultimate pH on the cumulative WL of the pork in these studies. All means reported are least square means. All animal procedures employed in this experiment were approved by the Iowa State University Committee on Animal Care.

Results

During the 4 h pre-transport feeding period, pigs consumed 342, 431, and 377g of the allocated feed in the 0, 2X, and 10X treatment groups resulting in quercetin intakes of 0, 1.07, and 4.68 mg, respectively (Table 2). These intakes are estimated to provide quercetin concentrations in the body water equivalent to 0, 1.5, and 7.1 times the K_i.

Dietary quercetin concentration, chill environment, and time postmortem each influenced postmortem muscle biochemistry (Table 3). As expected, the quick chill environment slowed postmortem muscle pH decline and lowered muscle temperature compared with the slow chill environment. Dietary quercetin slowed muscle pH decline at all points from 22 to 180 min postmortem. Specifically, quercetin consumption pooled across time postmortem, increased ($P < .10$) muscle pH in the quick chill (6.00, 6.08, and 6.07) and slow chill environments (5.79, 5.86, and 5.94), and the magnitude of response was greater ($P < .03$) in the slow chill environment. Dietary quercetin did not alter muscle temperature in the quick chill (30.7, 30.5, and 30.2°C) or slow chill environments (32.0, 32.1, and 32.1°C).

Dietary quercetin concentration, chill environment, and day of storage influenced pork quality traits. The quick chill environment resulted in a lower pork WL and Hunter L* color score (Table 4). The quick chill environment also lowered pork cooking loss but resulted in greater penetration resistance in the pork (Table 5). Dietary quercetin addition lowered ($P < .11$) pork WL, but the response was greater ($P < .05$) at the 2X versus 10X level of quercetin. The effect of dietary quercetin on pork color was influenced by chill environment and day of storage. Dietary quercetin addition raised ($P < .02$) Hunter L* values in the quick chill environment but lowered values in the slow chill environment. The magnitude of this interaction was minimized ($P < .10$) as storage progressed. Quercetin ingestion resulted in lower ($P < .01$) Hunter a* values, but the magnitude of change was minimized ($P < .11$) as storage time progressed. Dietary quercetin addition did not alter pork cooking loss or penetration resistance (Table 5).

Discussion

In this study, feeding the dietary glycolytic inhibitor quercetin decreased the rate of muscle pH decline and pork WL but did not alter ultimate pH. Some research suggests that ultimate pH is more important than is rate of pH decline for improving the WL of pork products (Kauffman et al., 1993). Other research indicates that the rate of pH decline rather than the ultimate pH affects the quality of subsequent pork products (Kremer et al., 1999). To further explore this relationship, a stepwise backward regression was performed to determine the relative impact of muscle temperature, rate of pH decline from 22 to 180 min postmortem, and ultimate pH on cumulative pork WL. Muscle temperature did not account for a significant amount of variation in this model. The R^2 for the model was .41 ($P < .01$). The ultimate pH accounted for .15 ($P < .01$) of the R^2, while the rate of pH decline accounted

for .18 ($P < .01$). The remaining portion of R^2 was accounted for by the interaction of pH decline and ultimate pH. Efforts to modify either or both of these parameters could have important effects on pork quality.

The fact that quercetin intake decreased the rate of pH decline and improved WL in both muscles, both chill temperatures, and across all time points observed in this experiment indicates that this technology is effective under a wide range of environmental conditions. The treatment of pigs with quercetin premortem would decrease some of the variability seen in pork quality, and the dietary inclusion of quercetin would lower the overall WL from pork products.

The ingestion of 9.6 and 43.9 μg of quercetin/kg BW in the 2X and 10X treatments slowed the rate of pH decline, which has been demonstrated to improve pork quality (Kremer et al., 1999). However, the 10X dosage of quercetin was less effective than the 2X dose at lowering pork WL in both chill environments. Perhaps the inhibition of glycolysis was great enough at the 10X dose to cause apoptosis of muscle cells in the pigs fed the higher dose of quercetin (Murray et al., 1996). These results indicate that the most effective dosage level of quercetin to improve water holding capacity in subsequent pork products likely is 9.6 μg of quercetin/kg BW.

The decrease in Hunter a* color score indicates that pigs consuming quercetin shortly before slaughter produced subsequent pork products with a less red color. This lower Hunter a* score would not be a positive attribute (NPPC, 1994). Generally, improved water holding capacity has been associated with increased soluble protein in the pork (Offer and Knight, 1988). In previous research (Kremer et al., 1999), ingestion of a glycolytic inhibitor that minimized postmortem pH decline and pork WL did alter pork Hunter L* during early

storage but did not alter Hunter a* color scores. In the present experiment, the Hunter a* color score was decreased by dietary quercetin addition, indicating that the pork was less red; however, this effect was minimized as storage time progressed.

The relationship between protein denaturization and color, and protein denaturization and WL has been described (Warner et al., 1997). Warner et al. (1997) have shown that increased binding of glycogen phosphorylase to sarcomeric protein is related to increased WL, while increased myoglobin denaturization is related to impaired color scores. The existence of the red, soft, and exudative (RSE) class of pork is evidence that color score and WL are not as closely related as they were thought to be (Offer and Knight, 1988; Warner et al., 1997).

Implications

Quercetin is a naturally occurring compound that inhibits glycolysis. Short term feeding of quercetin, provided in the aglycone form, is an effective technology for improving the water holding capacity of pork.

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Table 1. Basal diet composition (%)

Ingredient	% of Diet
Corn, yellow	82.445
Soybean meal, 48%	14.00
L-Lysine HCl	.20
Animal fat, stabilized	1.00
Dicalcium phosphate	.93
Limestone	.90
Salt	.25
TM-vitamin premix[a]	.25
Antimicrobial[b]	.025

[a] Contributed the following per kilogram of diet: vitamin A, 4405 IU; vitamin D_3, 1101 IU; vitamin E, 22 IU; niacin 33 mg; pantothenic acid, 17.6 mg; riboflavin, 6.6 mg; vitamin B_{12}, 22 μg; Fe, 88 mg; Zn, 75 mg; Cu, 8.8 mg; Mn, 30 mg; I, 1 mg.

[b] Contributed the following per kilogram of diet: bacitracin methylene disalicylate, 2 mg.

Table 2. Pig BW and feed intake during the 4 h pre-transport period.

	Dietary quercetin, K_i			
Criteria	**0**	**2X**	**10X**	**SEM**
Number of pigs	9	9	9	
Pig BW (kg)	109.1	112.6	106.4	1.26
Feed allocation (g/pig)	545	563	532	72
Feed intake (g/pig)	342	431	377	.08
Quercetin intake (mg/pig)	0	1.07	4.68	.06

Table 3. Effect of dietary quercetin on muscle pH and temperature postmortem (data pooled across muscle).

Criteria	Chill temperature	Time post-mortem	Dietary quercetin, K_i 0	2X	10X	SEM[a]
pH[b]	Quick	22 min	6.36	6.44	6.39	.11
		45 min	6.28	6.29	6.26	
		90 min	5.97	6.05	6.14	
		180 min	5.70	5.81	5.76	
		Mean	6.00	6.08	6.07	
	Slow	22 min	6.34	6.47	6.42	
		45 min	6.18	6.29	6.21	
		90 min	5.67	5.70	5.87	
		180 min	5.50	5.58	5.67	
		Mean	5.79	5.86	5.94	
Ultimate pH[c]	Quick	24 h	5.57	5.55	5.59	.05
	Slow	24 h	5.50	5.48	5.54	
Temperature, °C[d]	Quick	22 min	35.9	35.9	35.8	.06
		45 min	35.4	35.5	35.6	
		90 min	29.5	29.2	29.1	
		180 min	22.4	22.8	22.7	
		Mean	30.7	30.5	30.2	
	Slow	22 min	34.7	34.5	34.6	
		45 min	34.8	34.9	34.3	
		90 min	31.8	31.2	31.4	
		180 min	26.3	26.4	26.4	
		Mean	32.0	32.1	32.1	

[a] The SEM is calculated from the oxalate x chill temperature x muscle x period x replicate term.

[b] The pH was analyzed and means determined as hydrogen ion concentration and were converted for presentation, effect of quercetin 0 vs. 2X & 10X, $P < .10$; effect of chill temperature, $P < .01$; quadratic effect of time postmortem, $P < .01$; effect of dietary quercetin x chill temperature, $P < .03$; effect of chill temperature x time postmortem, $P < .01$.

[c] Effect of chill temperature, $P < .02$.

Table 3. Continued

[d] Effect of chill temperature, $P < .01$; effect of chill temperature x time postmortem, $P < .01$; quadratic effect of time postmortem, $P < .01$.

Table 4. The effect of dietary quercetin on pork water loss (WL) and color scores during storage (data pooled across muscle).

Criteria	Chill temperature	Time day of storage	Dietary quercetin, K_i 0	2X	10X	SEM[a]
WL, %[b]	Quick	0 to 3	7.5	5.8	6.7	.02
		3 to 6	10.0	8.3	9.1	
		6 to 9	11.7	9.9	10.9	
		9 to 12	12.6	10.9	12.0	
		Mean	9.9	8.1	9.8	
	Slow	0 to 3	9.9	7.0	8.3	
		3 to 6	12.6	9.4	10.6	
		6 to 9	14.2	11.0	12.3	
		9 to 12	15.2	12.0	13.1	
		Mean	12.8	10.1	12.1	
Hunter L*[c]	Quick	0	49.6	48.9	50.0	.14
		3	50.6	53.4	53.9	
		6	51.8	52.9	53.9	
		9	53.6	53.4	55.3	
		12	54.4	54.5	54.9	
		Mean	52.0	52.6	53.6	
	Slow	0	50.8	50.9	51.1	
		3	53.7	51.7	52.2	
		6	53.8	53.0	53.3	
		9	54.8	54.1	54.5	
		12	55.7	55.0	55.2	
		Mean	53.8	52.9	53.3	
Hunter a*[d]	Quick	0	5.5	5.0	4.6	.04
		3	6.8	6.0	5.8	
		6	6.1	5.3	5.4	
		9	5.1	4.8	4.6	
		12	4.2	4.2	4.0	
		Mean	5.5	5.1	4.9	

Table 4. Continued

Slow	0	6.3	4.8	5.0
	3	6.8	6.1	6.2
	6	5.8	5.4	5.2
	9	5.1	4.9	4.8
	12	4.4	3.8	4.3
	Mean	5.7	5.0	5.1

[a] The SEM is calculated from the oxalate x chill temperature x muscle x period x replicate term.

[b] Effect of dietary quercetin 0 vs. 2X & 10X, $P < .11$; effect of dietary quercetin 2X vs. 10X, $P < .05$; quadratic effect of days of storage, $P < .01$; effect of chill temperature, $P < .01$.

[c] Quadratic effect of days of storage, $P < .01$; effect of chill temperature, $P < .07$; effect of dietary quercetin x chill temperature, $P < .02$; effect of chill temperature x days of storage, $P < .03$; effect of dietary quercetin x chill temperature x days of storage, $P < .10$.

[d] Effect of dietary quercetin 0 vs. 2X &10X, $P < .01$; quadratic effect days of storage, $P < .01$; effect of dietary quercetin x days of storage, $P < .11$.

Table 5. Effect of dietary quercetin on pork cooking loss (CL) and penetration resistance (PR) (pooled across muscle)

Criteria	Chill temperature	Dietary quercetin K_i 0	2X	10X	SEM
CL, %[a]	Quick	29.4	29.9	29.2	.20
	Slow	29.6	30.7	30.8	
PR, kg[b]	Quick	3.04	2.84	2.94	.04
	Slow	2.92	2.80	2.76	

[a] Effect of chill temperature, $P < .14$.

[b] Effect of chill temperature, $P < .05$.

CHAPTER 5. THE EFFECT OF DIETARY VITAMIN C ON QUALITY OF PORK

A paper to be submitted to the *Journal of Animal Science*

B.T. Kremer[1], T.S. Stahly[1,2] and R.C. Ewan[1]

Abstract

Fourteen sets of three barrows, from a high lean halothane-negative genetic strain, weighing 29.7 ± 3.6 kg were used to determine the effects of dietary vitamin C on pork quality. Vitamin C can be metabolized to oxalic acid, which in turn has been shown to inhibit glycolysis and improve pork quality. Pigs were offered a basal diet (15 g/kg BW) containing 0, 783, and 2,348 ppm vitamin C, estimated to provide vitamin C amounts needed to produce body water concentrations of oxalate equivalent to 0, 2, and 6 times the K_i for pyruvate kinase, during a 4 h period before transport. Pigs were then transported 5 km to the ISU Meat Laboratory and killed within 60 min of arrival. Pigs were stunned, exsanguinated, and processed in an 18.3°C environment. Longissimus muscle pH and temperature were determined at 22, 45, 90, and 180 min postmortem. At 180 min postmortem, the carcass was chilled at 0°C. At 24 h postmortem, chops (2.5 cm) were taken from the longissimus and semimembranosus muscles for measurement of water loss (WL) and Hunter color scores on d 0, 3, 6, 9, and 12 of retail storage (5.6°C). Pigs consumed 325, 371, and 300 g of feed ($P < .05$), respectively, resulting in intakes of 0, 290, and 704 mg of vitamin C. Dietary vitamin C additions, pooled across time postmortem, increased ($P < .13$) muscle pH (6.18, 6.21, and 6.23). Dietary vitamin C addition at the high dose, pooled across days of storage, also lowered ($P < .15$) percentage pork WL (6.0, 5.8, and 5.2%) during storage. Dietary

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vitamin C additions also lowered ($P < .05$) Hunter L* values (48.7, 46.9, and 48.2), and raised ($P < .13$) Hunter a* color values (8.1, 8.8, and 8.4). Muscle pH and WL losses were similar between the 738 and 2,348 ppm feeding levels. Based on these data, short term feeding of vitamin C premortem is effective in slowing postmortem muscle pH decline and reducing the pork WL and improving the pork color scores.

Introduction

The traits and the relative importance of those traits to the quality of pork products has been reviewed (NPPC, 1994). Recent research suggests that inhibition of glycolysis may improve pork WL; however, results in relation to color scores has been inconsistent (Kremer et al., 1999ab). Inhibition of a glycolytic enzyme would decrease the rate at which lactic acid is formed. Oxalate inhibits muscle pyruvate kinase at a concentration (6 μM) lower than the level to inhibit liver (2.8 mM) or erythrocyte (0.27 mM) pyruvate kinase (Reed and Morgan, 1974; Buc et al., 1978). Oxalate improved the quality of pork when fed shortly prior to slaughter (Kremer et al., 1999a). Excess vitamin C is converted to oxalate (Tolbert et al., 1975). The efficiency of conversion of vitamin C to oxalate is approximately 5% (Horig, 1975).

The objective of the experiment was to determine whether high doses of vitamin C fed shortly before slaughter would minimize muscle pH decline postmortem and, in turn, improve pork quality.

Material and Methods

Fourteen sets of three barrows from a high lean, halothane negative genetic strain were used. Pigs were penned individually on a slotted floor in .61 m x 1.22 m pens in a thermal neutral environment (26.7°C). Before the experiment started, pigs were allowed ad

libitum access to a basal diet consisting of a corn and soybean meal mixture fortified with vitamins and minerals (Table 1). At a mean BW of 29.7 ± 3.6 kg, pigs were randomly allotted, within outcome groups based on BW, to one of three dietary treatments. The treatments consisted of the basal diet supplemented with 0, 783, or 2,348 ppm vitamin C. These supplemental levels of vitamin C were estimated to provide concentrations of oxalate, a vitamin C metabolite, in the body water equivalent to 0, 2, and 6 times the inhibitory constant (K_i) for pyruvate kinase. These concentrations are based on the following assumptions: 52.5% body water, absorption of 100% of dietary vitamin C (Tolbert et al., 1975), and the conversion of vitamin C to oxalate at an efficiency of 5% (Horig, 1975). Vitamin C, purity 99.9%, was obtained from Roche Vitamins, Inc. (616 Dayton Rd., Ames, Iowa 50010).

Each pig was allowed access to 15 g of feed/kg BW 4 h before loading and transport to the ISU Meat Laboratory. To enhance the consumption of the allocated feed (15 g/kg BW for 4 h before transport), pigs were removed from feed for 4 h (2300 to 0300 h) and then allowed ad libitum access to the allocated experimental feed for 4 h (0300 to 0700 h). Pigs were allowed ad libitum access to water throughout.

Pigs were transported (5 km) and killed within replicate groups within 60 min of arrival to the Iowa State University Meat Laboratory. Pigs were stunned for 7 s with 280 V, rolled onto their side to reduce catecholamine release (Troeger and Woltersdorf, 1989), and killed by exsanguination within 7 s of stunning. Pigs were not scalded. The head was removed and the carcass eviscerated within 10 min of stunning. Muscle pH and temperature were determined at 22, 45, 90, and 180 min postmortem. Muscle pH and temperature were measured in the right longissimus (LM) muscle by using an ISFET pH probe (Sentron 3001-

002; div. of Medtronic Synetics, 850 Victoria Street N, MS v215, Shoreview, MN 55126) and thermister. The pH probe and thermister are accurate to .02 pH units and .10°C, respectively. At 180 min postmortem, the carcass was chilled at 0°C. Muscle pH and temperature measurements were made in the right LM muscle posterior to the third, fourth, fifth, and sixth rib at 22, 45, 90, and 180 min postmortem, respectively.

At 24 h postmortem, the LM and semimembranosus (SM) muscles were cut into 2.5 cm chops (one WL chop and one color score chop), placed on absorbent pads, and wrapped with oxygen permeable over wrap. The LM and SM muscle chops were taken posterior to the 10^{th} and 11^{th} ribs and about 2.5 and 5.0 cm posterior to the anterior head of the SM muscle, respectively. Pork WL and color scores were determined on d 0, 3, 6, 9, and 12 d of storage. Pork WL was determined by unwrapping and weighing the same chop to the nearest mg on each designated day of storage. After weighing, the chop was placed on a new pad and re-wrapped in oxygen permeable over wrap. Pork color was determined by using the Hunter L*a*b* on a model JB-1201 M(A) Hunter LABSCAN machine (Hunter Assoc. Laboratory, Inc.; 11495 Sunset Hills road; Reston, VA 22090).

Data were analyzed by analysis of variance techniques by using the GLM procedure of SAS (1998). Muscle pH and temperature were analyzed as a randomized complete block design. Pork WL and color were analyzed as a split-plot design with dietary vitamin C considered the whole plot treatment and muscle type considered the split plot treatment. Responses over time were analyzed as repeated measures over time. Orthogonal contrasts were used to determine the effects of vitamin C consumption. All animal procedures employed in this experiment were approved by the Iowa State University Committee on Animal Care.

Results and Discussion

During the 4 h pre-transport feeding period, pigs consumed 325, 371, and 300 g of allocated feed, resulting in vitamin C intakes of 0, 290, and 704 mg, respectively (Table 2). These intakes are estimated to provide oxalate concentration in the body water equivalent to 0, 1.6, and 4.1 times the K_i for pyruvate kinase. Vitamin C consumption resulted in a greater ($P < .13$) muscle pH postmortem (6.18, 6.21, and 6.23) pooled across time postmortem, but the response increased as time progressed from 22 to 180 min postmortem (Table 3). The magnitude of the response was similar between the 2X and 6X levels of vitamin C supplementation. Dietary vitamin C did not alter muscle temperature (27.8, 27.6, and 27.8°C) pooled across time postmortem (Table 3).

Vitamin C consumption resulted in lower ($P < .15$) pork WL during storage (6.0, 5.8, and 5.2%, Table 4), and the magnitude of response was greater as length of storage progressed. The consumption of vitamin C lowered the Hunter L* (48.7, 46.9, and 48.2) and the magnitude of response was greater for the 2X versus the 6X level of vitamin C supplementation (Table 4). Vitamin C ingestion resulted in greater ($P < .13$) Hunter a* (8.1, 8.8, and 8.4, Table 4).

Previous research conducted on the efficacy of vitamin C for improving meat quality has not been successful (Fletcher and Cason, 1991). These researchers found that ingestion of vitamin C at rates similar to the 2X level fed in the present experiment (based on kg BW of each animal) did not improve the meat quality of broilers. The WL from the whole carcass and from the breast muscle was unchanged with vitamin C ingestion (Fletcher and Cason, 1991). In the present experiment, the timing of the dose of vitamin C was monitored closely to try to optimize the oxalate concentration in relation to slaughter. Fletcher and

Cason (1991) applied the vitamin C as a water treatment for 24 h before loading for slaughter. This may not have been the optimal timing for the consumption of vitamin C for this particular use.

In the current study, the inhibition of pH decline had a positive effect on the water loss of the subsequent product. Research has demonstrated that both the pH decline and ultimate pH are important to the subsequent pork WL (Kremer et al., 1999ab). Previous research has shown that slowing the rate of pH decline by short term feeding of glycolytic inhibitors did not alter the ultimate pH of pork (Kremer et al., 1999ab).

The lack of interaction between dietary vitamin C and muscle type on meat quality demonstrates that this dietary treatment was effective at improving pork quality under the different conditions that exist in the two muscle types monitored. The LM muscle chills more rapidly than the SM muscle (Kremer et al., 1999ab). The LM muscle is also more likely to have WL problems in market pigs than the SM muscle (Hedrick et al., 1994). Technology such as this would improve the overall quality of pork products.

The observations in this study indicated that vitamin C, or most likely its metabolite, oxalate, acted similarly to other glycolytic inhibitors that slowed the rate of muscle pH decline and improve the pork WL (Kremer et al., 1999ab). Feeding the vitamin C at the 6X level in this experiment lowered the pork WL by a magnitude similar to feeding sodium oxalate at a dosage estimated to provide oxalate at a level twice the K_i for pyruvate kinase (Kremer et al., 1999a). However, vitamin C feeding improved the color scores of subsequent pork products, whereas sodium oxalate did not (Kremer et al., 1999a). The glycolytic inhibitor quercetin also lowered WL but had either no effect or a detrimental effect on color scores (Kremer et al. 1999b).

This study demonstrated the effectiveness of vitamin C at lowering pork WL and improving color scores of pork. The validity of using a small BW pig in this study was previously demonstrated by the authors when evaluating the effect of sodium oxalate on pork quality (Kremer et al., 1999a). The authors demonstrated that the efficacy of sodium oxalate to improve pork quality was similar between 30 kg and 110 kg BW pigs.

Implications

Vitamin C can be converted to oxalate, which is a naturally occurring compound that inhibits glycolysis. Short term feeding of high concentration of vitamin C premortem is an effective technology for improving the water holding capacity and color scores of pork products.

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Table 1. Basal diet composition (%)

Ingredient	% of Diet
Corn, yellow	50.56
Whey, dried	10.00
Soybean meal, 48%	33.50
L-Lysine HCl	.20
DL Methionine	.10
Dicalcium phosphate	2.20
Limestone	.78
Salt	.25
TM-vitamin premix[a]	.41
Animal fat, stabilized	1.00
Antimicrobial[b]	1.00

[a] Contributed the following per kilogram of diet: vitamin A, 6608 IU; vitamin D_3, 1652 IU; vitamin E, 33 IU; niacin 50.7 mg; pantothenic acid, 26.4 mg; riboflavin, 9.9 mg; vitamin B_{12}, 33 μg; Fe, 105 mg; Zn, 90 mg; Cu, 10.5 mg; Mn, 36 mg; I, 1.2 mg; Se, .3 mg.

[b] Contributed following per kilogram of diet: 55 mg carbadox.

Table 2. Pig BW, feed intake, and vitamin C intake during the 4 h pre-transport period.

	Dietary vitamin C, K_i[a]			
Criteria	**0**	**2X**	**6X**	**SEM**
Number of pigs	14	14	14	
Pig BW, kg	29.6	30.3	29.2	.55
Feed allocation, g/pig	448	459	442	6
Feed intake, g/pig[b]	325	371	300	9
Vitamin C intake, mg/pig	0	290	704	13

[a] K_i are based on the estimated amount of oxalate produced by the metabolic conversion of vitamin C to oxalate.

[b] Effect of dietary vitamin C, 2X vs. 6X; $P < .05$.

Table 3. Effect of dietary vitamin C on muscle pH and temperature postmortem

Criteria	Time post mortem	Dietary vitamin C, K_i[a] 0	2X	6X	SEM
pH[b]	22 min	6.45	6.43	6.43	.02
	45 min	6.24	6.27	6.24	
	90 min	6.12	6.13	6.17	
	180 min	5.90	6.01	6.07	
	Mean	6.18	6.21	6.23	
Temperature, °C[c]	22 min	32.8	32.8	32.3	.37
	45 min	30.5	30.2	30.8	
	90 min	28.3	28.3	28.6	
	180 min	19.5	19.2	19.5	
	Mean	27.8	27.6	27.8	

[a] K_i are based on the estimated amount of oxalate produced by the metabolic conversion of vitamin C to oxalate.

[b] The pH was analyzed and means determined as hydrogen ion concentration and were converted for presentation, effect of dietary vitamin C, 0 vs. 2X & 6X, $P < .13$; linear effect of time $P < .01$; effect of dietary vitamin C x time postmortem, $P < .08$.

[c] Quadratic effect of time, $P < .01$.

Table 4. Effect of dietary vitamin C on pork water loss (WL) and color scores during storage (data pooled across muscle type)

Criteria	Day of storage	Dietary vitamin C, K_i[a] 0	2X	6X	SEM[b]
WL, %[c]	0 to 3	2.9	3.0	2.5	.12
	3 to 6	5.3	5.1	4.6	
	6 to 9	7.1	6.8	6.2	
	9 to 12	8.6	8.2	7.6	
	Mean	6.0	5.8	5.2	
Hunter L*[d]	0	45.8	44.1	45.4	.08
	3	47.9	45.7	47.5	
	6	49.6	47.9	49.0	
	9	49.5	47.4	48.6	
	12	50.9	49.2	50.3	
	Mean	48.7	46.9	48.2	
Hunter a*[e]	0	6.8	7.7	7.2	.05
	3	8.9	10.0	9.2	
	6	8.8	9.5	9.2	
	9	8.4	9.2	8.8	
	12	7.3	7.6	7.4	
	Mean	8.1	8.8	8.4	

[a] K_i are based on the estimated amount of oxalate produced by the metabolic conversion of vitamin C to oxalate.

[b] The SEM is calculated from the oxalate x muscle x period x replicate term.

[c] Effect of dietary vitamin C, 0 vs. 2X & 6X, $P < .15$; quadratic effect of days of storage, $P < .05$; effect of dietary vitamin C x days of storage, $P < .05$.

[d] Effect of dietary vitamin C, 0 vs. 2X & 6X, $P < .05$; effect of dietary vitamin C 2X vs. 6X, $P < .05$; quadratic effect of days of storage, $P < .01$.

[e] Effect of dietary vitamin C, 0 vs. 2X & 6X, $P < .13$; quadratic effect of days of storage, $P < .01$.

CHAPTER 6. EFFECT OF DIETARY ZINC ON PORK QUALITY

A paper to be submitted to the *Journal of Animal Science*

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Abstract

Fourteen sets of four barrows, from a high lean, halothane-negative genetic strain, weighing 30.9 ± 3.0 kg were used to determine the effects of dietary zinc on pork quality. Zinc has been shown to inhibit phosphofructokinase with a K_i of 200 μM. Pigs were offered a basal diet (15 g/kg BW) containing 0, 14, 28, or 42 g zinc oxide/kg of feed for the 4 h period before transport. Pigs were then transported 5 km to the ISU Meat Laboratory and killed within 60 min of arrival. Pigs were stunned with 270 V for 5 s and exsanguinated. Longissimus muscle pH and temperature were determined at 22, 45, 90, and 180 min postmortem. At 180 min postmortem, the carcass was chilled at 0°C. At 24 h postmortem, chops (2.5 cm) were taken from the longissimus and semimembranosus muscles for measurement of water loss (WL) and Hunter color scores on d 0, 3, 6, 9, and 12 of retail storage (5.6°C). Pigs consumed 340, 348, 278, and 280 g of feed ($P < .03$), respectively, resulting in intakes of 0, 4.86, 7.78, and 11.75 g of zinc oxide. Dietary zinc addition did not ($P > .15$) alter postmortem muscle pH (6.11, 6.18, 6.15, 6.19) pooled across time postmortem. Dietary zinc additions also did not ($P > .15$) affect percentage WL (9.5, 9.2, 8.8, 9.0%) pooled across storage days. Dietary zinc addition did not ($P > .15$) alter Hunter L* values (50.4, 50.9, 50.2, 50.3) but tended to linearly raise ($P < .15$) Hunter a* color values

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(7.3, 6.8, 7.6, 7.6). Short term feeding of high concentrations zinc, a glycolytic inhibitor, was ineffective in reducing the pork WL but tended to improve pork redness.

Introduction

The quality concerns for pork have been discussed (NPPC, 1994). Inhibition of glycolysis via short term feeding of glycolytic inhibitors effectively reduced postmortem muscle pH decline and pork water loss (WL) during storage (Kremer et al., 1999abc). The effects of dietary glycolytic inhibitors on pork color have been inconsistent (Kremer et al., 1999abc). The trace mineral zinc, when present at high (10 μM, equivalent to K_i) has been shown to inhibit phosphofructokinase (Ikeda et al., 1980). The concentration of zinc required to K_i for phosphofructokinase in the presence of histidine is 200 μM (Ikeda et al., 1980).

The objective of this study was to determine the effect of high doses of dietary zinc shortly prior to slaughter on postmortem muscle pH decline and subsequent quality characteristics of pork.

Material and Methods

Fourteen sets of four barrows from a high lean, halothane-negative genetic strain were penned individually in .61 m x 1.22 m pens in a thermal neutral environment (26.7°C). Pigs were allowed ad libitum access to a basal corn-soybean meal diet supplemented with 90 ppm zinc (Table 1). At a body weight of 30.9 ± 3.0 kg, four pigs were randomly allotted, outcome groups based on BW, to one of four dietary treatments. The treatments consisted of the basal diet supplemented with 0, 14, 28, or 42 g zinc oxide/kg feed. These supplemental zinc additions were estimated to provide body water zinc concentrations equivalent to 0, 1, 2, and 3 times the K_i of zinc (200 μM) for muscle phosphofructokinase (Ikeda et al., 1980). The concentrations were estimated from the following assumptions: pigs were assumed to

contain 52.5% body water, the absorption was assumed to be 5% (Hill et al., 1987) for the calculation, and distribution was assumed to be complete in body water. At 30.9 ± 3.0 kg, pigs were removed from feed for 4 h (2300 to 0300 h) and then allowed ad libitum access to the allocated experimental feed for 4 h (0300 to 0700 h). Zinc oxide (Prince Agriproducts, Inc., One Prince Plaza, Quincy, IL 62301), 72% purity, was used as the dietary source of supplemental zinc. At 0700, pigs were gently loaded and then transported 5 km to the Iowa State University Meat Laboratory.

The slaughter, processing, and sampling were identical to those used by Kremer et al. (1999c). Muscle pH and temperature were measured at 22, 45, 90, and 180 min postmortem on the right longissimus (LM) muscle. The ultimate pH was measured at 24 h postmortem in the right longissimus muscle. Muscle pH and temperature were measured in the right LM muscle by using an ISFET pH probe (Sentron 3001-002; div. of Medtronic Synetics, 850 Victoria Street N, MS v215, Shoreview, MN 55126) and thermister. The pH probe and thermister are accurate to .02 pH units and .10°C, respectively. At 180 min postmortem, the carcass was chilled at 0°C. Muscle pH and temperature measurements were made in the right LM muscle posterior to the third, fourth, fifth, and sixth rib at 22, 45, 90, and 180 min postmortem, respectively.

At 24 h postmortem, the LM and semimembranosus (SM) muscles were cut into 2.5 cm chops (one WL chop and one color score chop), placed on absorbent pads, and wrapped with oxygen permeable over wrap. The LM and SM chops were taken posterior to the 10^{th} and 11^{th} ribs and about 2.5 and 5.0 cm posterior to the anterior head of the SM muscle, respectively. Pork WL and color scores were determined on day 0, 3,6, 9, and 12 d of storage. Pork WL was determined by unwrapping and weighing the same chop to the nearest

milligram on each designated day of storage. After weighing, the chop was placed on a new pad and re-wrapped in oxygen permeable over wrap. Pork color was determined by using the Hunter L*a*b* on a model JB-1201 M(A) Hunter LABSCAN machine (Hunter Assoc. Laboratory, Inc.; 11495 Sunset Hills road; Reston, VA 22090).

Zinc was determined in the muscle tissue by a nitric acid and perchloric acid digestion (Johnson and Ulrich, 1959) followed by dilution and quantitation via atomic absorption (Perkin Elmer 3100; 761 Main Ave.; Norwalk, CT 06859) as outlined by Ewan et al. (1970).

Data were analyzed by analysis of variance techniques by using the GLM procedure of SAS (1998). Dietary zinc concentration was considered the whole plot treatment and muscle type the sub plot treatment. Response over time was analyzed as repeated measures. Orthogonal comparisons were made to determine the linear, quadratic, and cubic effects of dietary zinc concentration. The least square means are reported.

All animal procedures were approved by the Iowa State University Committee on Animal Care.

Results and Discussion

During the 4 h pre-transport feeding period, pigs consumed 340, 348, 278, and 280 g of feed in the 0, 1 X, 2X, and 3X treatment groups, respectively, resulting in zinc oxide intakes of 0, 4.86, 7.78, and 11.75 g (Table 2). These intakes are estimated to provide 0, .75, 1.18, and 1.83 times the K_i for muscle phosphofructokinase. Zinc consumption did not ($P > .15$) alter muscle pH (6.11, 6.18, 6.15, 6.19) or muscle temperature (30.0, 29.7, 30.0, 29.7°C; $P > .15$, Table 3). Zinc consumption did not ($P > .15$) alter pork WL (9.5, 9.2, 8.8, 9.0%) during storage (Table 4). The consumption of zinc did not alter ($P > .15$) the Hunter L*

(50.4, 50.9, 50.2, 50.3) but tended to linearly ($P < .15$) raise Hunter a* (7.3, 6.8, 7.6, 7.6, Table 4). The level of zinc in the meat was not altered ($P > .15$) by the dietary treatment (11.71, 12.11, 14.78, 13.82 ppm, Table 4).

Because dietary zinc oxide additions did not alter postmortem muscle pH decline and pork WL, the trend for increased pork redness with added zinc oxide was not expected. Faster rates of muscle pH decline are associated with color and WL problems (Offer and Knight, 1988). The observations of different classes of pork, red, soft, and exudative (RSE), pale, soft, and exudative (PSE), and normal (Warner et al., 1996) may indicate that color score and WL are not as closely related as once thought. It has also been demonstrated that supplementation with a divalent ion, magnesium, improved the Hunter L* color score (D'Souza et al., 1998).

The decision to include zinc at levels estimate to provide 0, 1, 2, and 3 times the K_i was based on the amount of zinc required to obtain these dietary levels. The lack of effect of dietary zinc, a glycolytic inhibitor, in relation to pork quality was not expected because short term feeding of glycolytic inhibitors at estimated inhibition concentrations of two times the K_i lowered WL in pork products (Kremer et al., 1999abc). The dietary zinc intakes observed in the study resulted in lower tissue zinc concentration than expected from the assumptions made to calculate the zinc oxide dose. Tissue zinc levels, assuming a 5% absorption, complete mixing in body fluids, and 52.5% body water were expected to increase zinc 8.5, 17.0, and 25.5 ppm, over the basal pig for the 1X, 2X, and 3X levels of supplementation, respectively. However, the muscle zinc level was altered only .4, 2.6, and 2.1 ppm, respectively (Table 4). There would seem to be two possible reasons for the lack of response of muscle Zn concentration to dietary zinc inclusion. Either the absorption value of 5% for

absorption was in error, or the zinc was sequestered in the liver (Cousins, 1979). Lewis (1957) reported that muscle tissue did not increase zinc level with increased dietary zinc intakes up to 1,028 ppm. The absorption of zinc can vary widely based on the source of the zinc oxide (Underwood, 1971). The value of 5% was used as a best estimate from several sources (Underwood, 1971; Hill et al., 1987). Zinc can also be bound in the liver by metallothionein (Cousins, 1979). Binding of zinc by the metallothionein in the liver would not allow zinc to accumulate in the muscle tissue. If the glycolytic inhibitor was not present in the muscle at sufficient concentrations, the site of lactic acid production, then the inhibitor will not be expected to inhibit glycolysis and the resultant rate of pH decline. The lack of effect on the water holding would be expected in this case because there was little effect of zinc on the pH decline as measured in this study.

Implications

The dietary inclusion of high concentrations of zinc shortly before slaughter is not an effective technique for improving the pH decline and water loss of pork. Because of the positive effect of Zn on color score, this treatment potentially could have value when paired with other orally active glycolytic inhibitors to improve both WL and color score.

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Table 1. Basal diet composition (%)

Ingredient	% of Diet
Corn, yellow	50.56
Whey, dried	10.00
Soybean meal, 48%	33.50
L-Lysine HCl	.20
Animal fat, stabilized	1.00
DL Methionine	.10
Dicalcium phosphate	2.20
Limestone	.78
Salt	.25
TM-vitamin premix[a]	.41
Antimicrobial[b]	1.00

[a] Contributed the following per kilogram of diet: vitamin A, 6608 IU; vitamin D_3, 1652 IU; vitamin E, 33 IU; niacin, 50.7 mg; pantothenic acid, 26.4 mg; riboflavin, 9.9 mg; vitamin B_{12}, 33 μg; Fe, 105 mg; Zn (from zinc oxide), 90 mg; Cu, 10.5 mg; Mn, 36 mg; I, 1.2 mg; Se, .3 mg.

[b] Contributed the following per kilogram of diet: carbadox, 55mg.

Table 2. Pig BW and feed intakes

	Zinc, K_i				
Criteria	**0**	**1X**	**2X**	**3X**	**SEM**
Number of pigs	14	14	14	14	
Pig BW, kg	31.6	30.9	31.1	30.5	.35
Feed allocation, g/pig	470	467	470	461	13
Feed intake, g/pig[a]	340	348	278	280	5
Zinc oxide intake, g/pig	0	4.9	7.8	11.8	.37

[a] Linear effect of dietary zinc, $P < .03$.

Table 3. Effect of dietary zinc concentration on muscle pH and temperature postmortem

Criteria	Time post mortem	Zinc, K_i 0	1X	2X	3X	SEM
pH[ab]	22 min	6.36	6.43	6.40	6.50	.02
	45 min	6.25	6.27	6.26	6.32	
	90 min	6.03	6.14	6.12	6.10	
	180 min	5.79	5.89	5.84	5.83	
	Mean	6.11	6.18	6.15	6.19	
Ultimate pH	24 h	5.74	5.75	5.73	5.76	.01
Temperature, °C[a]	22 min	34.2	34.3	34.4	33.7	.23
	45 min	31.7	31.3	31.5	31.4	
	90 min	28.4	27.6	28.1	28.1	
	180 min	25.6	25.4	26.0	25.6	
	Mean	30.0	29.7	30.0	29.7	

[a] Quadratic effect of time postmortem, $P < .03$.

[b] The pH was analyzed and means determined as hydrogen ion concentration and were converted for presentation.

Table 4. Effect of dietary zinc on pork water loss WL and Hunter scores during storage (data pooled across muscle type)

Criteria	Day of storage	Zinc, K_i 0	1X	2X	3X	SEM[a]
WL, %[b]	0 to 3	6.4	6.2	5.8	6.0	.17
	3 to 6	9.0	8.7	8.3	8.5	
	6 to 9	10.7	10.3	10.0	10.2	
	9 to 12	11.9	11.5	11.1	11.4	
	Mean	9.5	9.2	8.8	9.0	
Hunter L*[b]	0	47.1	47.9	47.1	46.9	.07
	3	50.2	50.6	49.9	50.0	
	6	51.2	51.6	51.0	51.1	
	9	51.2	51.9	51.3	51.4	
	12	52.1	52.6	51.9	52.0	
	Mean	50.4	50.9	50.2	50.3	
Hunter a*[bc]	0	7.4	6.4	7.3	7.3	.04
	3	8.8	8.2	9.0	9.0	
	6	8.3	7.8	8.5	8.5	
	9	7.0	6.5	7.4	7.6	
	12	5.1	5.1	5.5	5.7	
	Mean	7.3	6.8	7.6	7.6	
Muscle zinc (ppm)		11.71	12.11	14.78	13.82	1.13

[a] The SEM is calculated from the oxalate x muscle x period x replicate term.
[b] Quadratic effect of day of storage, $P < .02$.
[c] Linear effect of dietary Zn, $p < .15$.

CHAPTER 7. GENERAL CONCLUSIONS

The inclusion of dietary glycolytic inhibitors shortly before slaughter decreased the rate of muscle pH decline postmortem and lowered pork WL. The response seen in the pork color scores were inconsistent in that the color scores were improved in some cases, not affected in others, and detrimentally altered in other cases. The inhibitors investigated in this series of experiments were oxalate, quercetin, vitamin C, and zinc. Oxalate inhibits the glycolytic enzyme, pyruvate kinase. Quercetin inhibits the glycolytic enzyme, lactate dehydrogenase. Vitamin C can be metabolized to oxalate, which inhibits pyruvate kinase. Zinc inhibits the glycolytic enzyme, phosphofructokinase.

Sodium oxalate consumption lowered the rate of pH decline in experiment one ($P < .06$) independent of time, chill temperature, and muscle type. In experiment two, the postmortem muscle pH decline was again decreased, but the magnitude of the response was greatest at 180 min postmortem. The subsequent pork products had lower WL in experiment one, and the magnitude of response was constant in the quick chill environment but increased in magnitude in the slow chill environment as days of storage progressed. In experiment two, the dietary sodium oxalate lowered WL independent of days of storage. Pork consumption of dietary sodium oxalate did not alter pork color scores. Quercetin decreased the rate of pH decline in the postmortem muscle ($P < .10$), and the magnitude of the response was greater in the slow chill environment. Ingestion of quercetin also lowered the pork WL during storage ($P < .11$), and the magnitude of the response was greater ($P < .05$) at the 2X level versus the 10X level of quercetin. Dietary quercetin also raised Hunter L* in the quick chill environment, but lowered Hunter L* in the slow chill environment. Hunter a* color score was decreased ($P < .01$) with dietary quercetin, the magnitude of this change was

decreased as storage progressed. Inclusion of high doses of vitamin C decreased ($P < .13$) the rate of pH decline in the postmortem muscle, but the magnitude of the response increased as time progressed from 22 to 180 min postmortem. Vitamin C consumption resulted in lower ($P < .15$) pork WL, and the magnitude of the response was greater as day of storage progressed. Hunter L* was lowered ($P < .05$) with vitamin C consumption and the magnitude of the response was greater for the 2X versus the 6X dose. Hunter a* was increased ($P < .13$) by vitamin C supplementation. Dietary inclusion of high doses of Zn oxide did not alter the rate of muscle pH decline or the pork WL. Dietary zinc inclusion did tend to linearly increase Hunter a* color scores ($P < .15$) independent of time and muscle type.

Inclusion of a dietary glycolytic inhibitor 4 h before transport to the slaughter plant is an effective technology for decreasing the rate of postmortem muscle pH decline. Ingestion of glycolytic inhibitors also increased subsequent pork WL.